

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE #12

In Re : U.S. Patent No. 4,883,812
Issued : November 28, 1989
Inventors : Salvador Moncada
Assignee : Burroughs Wellcome Co.
For : TREATMENT OF HYPERTENSION USING PROSTACYCLIN

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OFFICE OF PETITIONS
AND PATENTS

Commissioner of Patent and Trademarks
Box Patent Extension
Washington, DC 20231

APPLICATION FOR PATENT TERM EXTENSION UNDER 35 U.S.C. 156

P 30021 11/21/95 4883812

02-4857 030 111 1,060.00CH

Sir:

Applicant, Glaxo Wellcome Inc., a corporation of the State of North Carolina and successor in interest to Burroughs Wellcome Co. by virtue of merger and change of name, represents pursuant to 35 U.S.C. 156(d)(1), that it is the record owner and assignee of the entire interest in and to Letters Patent of the United States of America No. 4,883,812 granted to Salvador Moncado on November 28, 1989 for TREATMENT OF HYPERTENSION USING PROSTACYCLIN by virtue of an assignment to Burroughs Wellcome Co. recorded in the United States Patent and Trademark Office on December 7, 1977, Reel 3479, Frames 683-686. A true copy of said assignment and Certificate of Merger / Change of Name are attached hereto as EXHIBITS 1 & 2, respectively.

Applicant further represents, pursuant to 37 C.F.R. 1.785(d), that it is the holder of the regulatory approval granted by the Food and Drug Administration ("FDA") for FLOLAN® (epoprostenol sodium) for Injection (hereinafter, "FLOLAN® Injection"). See EXHIBIT 4.

Applicant presents this Application for Extension of Patent Term under 35 U.S.C 156 according to the format set forth in 37 C.F.R. 1.740(a).

Applicant hereby submits this application, in the alternative with an application for extension of U.S. Patent 4,338,325, both in respect of FLOLAN® Injection, duly electing U.S. Patent 4,883,812 to be extended and in the alternative U.S. Patent 4,338,325 pursuant to 37 C.F.R. 1.785(b). Applicant makes known that the present election is not intended to waive any right of appeal should its election or alternative be denied a term extension.

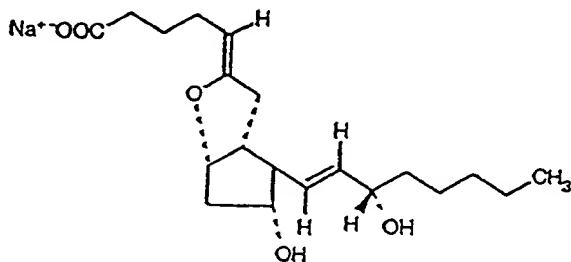
- (1) This application for extension is based upon the regulatory review period before the FDA of Applicant's approved product, FLOLAN[®] Injection. The only active ingredient in FLOLAN[®] Injection is epoprostenol sodium. A copy of the labeling approved by the FDA as part of New Drug Application ("NDA") 20-444 for the approved product is attached hereto as EXHIBIT 5. Identification of the approved product, FLOLAN[®] Injection, is provided as follows:

Chemical Name: Epoprostenol is (5*Z*,9,11,13*E*,15*S*)-6,9-epoxy-11,15-dihydroxyprosta-5,13-dien-1-oic acid.

The active ingredient is epoprostenol sodium.
(as per approved labeling, see EXHIBIT 5)

Molecular Formula: $C_{20}H_{31}NaO_5$

Structural Formula:



Molecular Weight: 374.45 Daltons

Description: FLOLAN[®] (epoprostenol sodium) for Injection is a sterile sodium salt formulated for intravenous administration.

FLOLAN[®] is a white to off-white powder that must be reconstituted with STERILE DILUENT for FLOLAN[®].

After being reconstituted, each vial of FLOLAN[®] contains epoprostenol sodium equivalent to either 0.5 mg or 1.5 mg epoprostenol, 3.76 mg glycine, 2.93 mg sodium chloride, 50 mg mannitol and Water for Injection, USP. Sodium hydroxide may have been added to adjust the pH between 10.2 and 10.8. See EXHIBIT 5.

- (2) The approved product, FLOLAN® Injection, was subject to regulatory review under Federal Food, Drug and Cosmetic Act, section 505 (21 U.S.C. 355).
- (3) FLOLAN® Injection received permission for commercial marketing or use under section 505 of the Federal Food, Drug and Cosmetic Act (21 U.S.C. 355) on September 20, 1995. See EXHIBIT 4.
- (4) Epoprostenol sodium, the only active ingredient in FLOLAN® Injection, has not been previously approved for commercial marketing under the Federal Food Drug and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act.
- (5) This application for extension of patent term under 35 U.S.C. 156 is being submitted within the permitted 60 day period, which will expire on November 18, 1995.
- (6) The complete identification of the patent for which extension of term is being sought is as follows:

U.S. Patent	:	4,883,812
For	:	TREATMENT OF HYPERTENSION USING PROSTACYCLIN
Inventors	:	Salvador Moncado
Assignee	:	Burroughs Wellcome Co.
Issue Date	:	November 28, 1989
Expiration Date (as terminally disclaimed against U.S. 4,539,333)	:	September 3, 2002

- (7) A complete copy of the patent identified in paragraph (6) above is appended hereto as EXHIBIT 6.
- (8) (a) Copies of the receipts of all maintenance fee payments made with respect to U.S. Patent 4,883,812 are attached hereto as EXHIBIT 3.
- (b) A copy of the Terminal Disclaimer is attached hereto as EXHIBIT 12.
- (c) No certificate of correction or reexamination certificate exists in respect of U.S. Patent 4,883,812.

- (9) United States Patent Number 4,883,812 claims a method of using the active ingredient in the approved product FLOLAN® Injection. Applicant hereinbelow lists each applicable patent claim and demonstrates the manner in which each applicable patent claim reads on the method of using the approved product.
- (a) Claim 3 reads as follows: "A method of treating a mammal for hypertension which comprises parenterally administering an effective antihypertensive amount of prostacyclin anion in a liquid to said mammal."
- (1) The approved product, FLOLAN® (epoprostenol sodium) for Injection is an injectable solution containing a liquid and the salt, epoprostenol sodium.
- (a) Epoprostenol sodium is prostacyclin sodium. See EXHIBIT 13.
- (b) Since the salt of prostacyclin contains the prostacyclin anion (the prostacyclin molecule with a negatively charged carboxyl moiety) associated with a cation, *e.g.*, sodium, claim 3 reads on the approved product since it claims a method of using "prostacyclin anion" for treating hypertension.
- (2) The approved product, FLOLAN® Injection, is indicated for the long-term intravenous treatment of primary pulmonary hypertension in NYHA Class III and Class IV patients.
- (a) Primary pulmonary hypertension is a type of hypertension.
- (b) 9.2 ng/kg/min is an effective antihypertensive amount of prostacyclin anion.
- (c) The approved product is administered intravenously which is a type of parenteral administration.
- (d) NYHA Class III and Class IV patients belong to the class *Mammalia*.
- (b) Claim 4 reads as follows: "A method of claim 3 in which the prostacyclin anion is administered by injection."
- (1) The approved product is administered intravenously which is accomplished by injection.

(10) The relevant dates and information pursuant to 35 U.S.C. 156(g) necessary to enable the Secretary of Health and Human Services to determine the applicable regulatory review period are as follows:

(a) Effective Date of IND

The Investigational New Drug ("IND") application for FLOLAN® Injection was filed May 30, 1979 and assigned IND number 16,459. The IND became effective 30 days thereafter on June 29, 1979.

(b) Issue Date of Patent

U.S. Patent No. 4,883,812 issued November 28, 1989 and claims a method of using new a drug.

(c) Submission Date of NDA

The NDA for FLOLAN® Injection was submitted February 28, 1994 and assigned NDA number 20-444.

(d) Approval Date of NDA

NDA 20-444 for FLOLAN® Injection was approved by the FDA on September 20, 1995.

- (11) A brief description of each significant activity undertaken by Applicant during both the IND and NDA regulatory periods is presented in chronological form and is attached hereto as EXHIBIT 7, "Due Diligence Log".
- (a) The Due Diligence Log reflects significant communications between Applicant and FDA during regulatory periods. Such communications include, but are not limited to: submission of pre-clinical reports; registration of clinical protocols and amendments thereof; registration of clinical investigators and amendments thereof; submission of adverse event reports; submission of IND Annual Reports, etc.
 - (b) Periods between such communications enumerated in the Due Diligence Log reflect Applicant's diligent undertaking of the necessary clinical studies and other activities required by the FDA in order to obtain approval for Applicant's product.

(12) Applicant is of the opinion that U.S. Patent 4,883,812 is eligible for a 1346 day extension pursuant to 35 U.S.C. 156(g)(6)(A).

(a) Applicant has satisfied the eligibility criteria necessary to obtain a patent term extension pursuant to 35 U.S.C. 156.

(1) 35 U.S.C 156(a)

U.S. Patent 4,883,812 claims a method of using a drug product.

(2) 35 U.S.C. 156(a)(1)

The term of U.S. Patent 4,883,812 has not expired before submission of this application.

(3) 35 U.S.C. 156(a)(2)

The term of U.S. Patent 4,883,812 has never been extended.

(4) 35 U.S.C. 156(a)(3)

The application for extension is submitted by the owner of record in accordance with the requirements of 35 U.S.C. 156(d) and 37 C.F.R. 1.710 *et seq.*

(5) 35 U.S.C. 156(a)(4)

The approved product, FLOLAN[®] Injection, has been subject to a regulatory review period before its commercial marketing or use.

(6) 35 U.S.C. 156(a)(5)(A)

The commercial marketing or use of the approved product, FLOLAN[®] Injection, after the regulatory review period is the first permitted commercial marketing or use of the approved product under the provisions of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 355) under which such regulatory review period occurred.

(b) Applicant herewith, claims a patent term extension of 1346 days for U.S. Patent 4,883,812 pursuant to 35 U.S.C. 156(g) as follows:

(1) One half of the IND regulatory review period for the approved product beginning November 28, 1989 (the IND period continuing from the date of the issuance of U.S. Patent 4,883,812) and ending on February 27, 1994 (one day prior to the date on which the NDA for the approved product was initially submitted), such sum being equal to 776 days.

(2) The full term of the NDA regulatory review period commencing February 28, 1994 (the date NDA 20-444 for the approved product was originally submitted) and ending on September 20, 1995 (the date on which NDA 20-444 was approved), such sum being equal to 570 days. See EXHIBIT 8.

(3) The sum of paragraphs (1) and (2) in this subsection equals 1346 days. Said sum of 1346 and does not exceed the maximum allowable extension of 5 years pursuant to 35 U.S.C. 156(g)(6)(A). See EXHIBIT 8.

- (c) Applicant herewith, claims an expiration date of May 11, 2006 for U.S. Patent 4,883,812 pursuant to 35 U.S.C. 156(c)(3).
- (1) The expiration of U.S. Patent 4,883,812, as terminally disclaimed against U.S. 4,539,333 is September 3, 2002.
 - (2) Extending the September 3, 2002 expiration by 1346 days would result in an expiration date of May 11, 2006.
 - (3) Being that expiration of U.S. Patent 4,883,812 believed to be entitled to said 1346 day extension is May 11, 2006, the limitation in 35 U.S.C. 156(c)(3) which requires that term extensions be reduced in order to limit the expiration date of a patent receiving term extensions to 14 years from the date of NDA approval is not reached, since 14 years from the date of NDA approval is September 20, 2009. See EXHIBIT 8.
- (13) The Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to any determinations to be made relative to the application for extension. In this regard, Applicant wishes to disclose the following:
- (a) In the late 1970's, an interference was declared by the USPTO between the application which led up to Moncada U.S. Patent 4,539,333 and one of the earlier applications which led up to the Upjohn (Johnson *et al.*) U.S. Patent 4,338,325.
 - (1) Both parties were contesting the priority rights related to the count which covered the inventions in both applications. However in the USPTO, it was decided that the claims present in the Moncada application to prostacyclin *per se* were to a product of nature and therefore were held unpatentable to Moncada and the interference was dissolved and patents issued to the respective parties.
 - (2) Upjohn, to the best of Applicant's knowledge, had a U.S. application in the interference with an earlier date as to the salts of epoprostenol, whereas the first UK priority application of the Moncada disclosure was limited to prostacyclin *per se*. Upjohn did not claim prostacyclin *per se* since that was not their invention.
 - (3) Subsequently, the Moncada application leading up to U.S. Patent 4,883,812 was filed for treating hypertension.
 - (b) With respect to the Johnson patent 4,338,325 cited in 4,883,812, there was no disclosure in Johnson '325 of the use of prostacyclin anion in the treatment of hypertension. Treatment of high blood pressure (hypertension) is based on the vasodilatory action of prostacyclin and its salts. (U.S. 4,883,812 Col.4, lines 24-30). At Col. 5, lines 33-35 of U.S. 4,883,812, there is disclosed liquid carriers.
 - (c) In addition to enclosing the above mentioned patents, Applicant attaches U.S. 4,335,139 which claims the formulation of the approved product. See EXHIBITS 9-11.

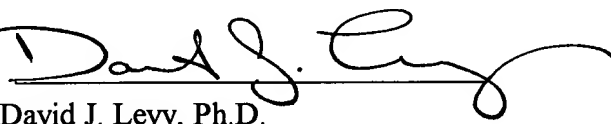
- (14) The Commissioner of Patents and Trademarks is authorized to charge deposit account 02-4857 in the amount of \$1,030.00 for receiving and acting upon this application for extension of term. In the event the actual fee differs from that specified above, it is requested that the overpayment be charged or the underpayment credited as authorized in the letter from David J. Levy, Ph.D. enclosed herewith.
- (15) Inquiries and correspondence relating to this application for patent term extension are to be directed to:

David J. Levy, Ph.D.
Patent Counsel, Glaxo Wellcome Inc.
Five Moore Drive
Research Triangle Park, NC 27709
(919) 248-7656

- (16) A duplicate of the application papers, certified as such is attached hereto.
- (17) Submitted herewith is a Declaration by David J. Levy, Ph.D., Patent Counsel for Glaxo Wellcome Inc., which meets the criteria set forth in 37 C.F.R. 1.740(b).

The undersigned hereby certifies that this Application for Extension of Patent Term Under 35 U.S.C. 156 including its EXHIBITS and supporting papers is being submitted as duplicate originals.

Respectfully submitted,
Glaxo Wellcome Inc.

By: 
David J. Levy, Ph.D.
Patent Counsel

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re : U.S. Patent No. 4,883,812
Issued : November 28, 1989
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DECLARATION UNDER C.F.R. 1.740(b)

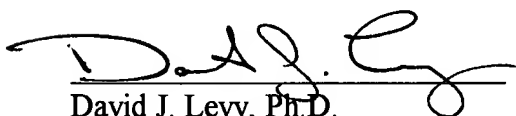
To the Commissioner of Patents and Trademarks:

I, David J. Levy, residing in Raleigh, North Carolina, declare as follows:

- (1) That I am a patent attorney authorized to practice before the United States Patent and Trademark Office and that my registration number is 27,655.
- (2) That I make this declaration as Patent Counsel for Glaxo Wellcome Inc., a corporation of the State of North Carolina and successor in interest to Burroughs Wellcome Co. by virtue of merger and name change, having a place of business at Five Moore Drive, Research Triangle Park, North Carolina 27709 and have general authority to act on its behalf in patent matters.
- (3) That Burroughs Wellcome Co. was the assignee of the entire right, title and interest in United States Patent 4,883,812 issued November 28, 1989 (hereinafter "Patent") having now been succeeded in interest by Glaxo Wellcome Inc., by virtue of merger and name change.
- (4) That I have reviewed and understand the contents of the APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. 156 submitted herewith on behalf of Glaxo Wellcome Inc. requesting a 1346 day extension of the term of the Patent.

- (5) That said application is being submitted in the alternative with an application for extension of U.S. Patent 4,338,325, both in respect of FLOLAN® (epoprostenol sodium) for Injection, duly electing U.S. Patent 4,883,812 to be extended and in the alternative U.S. Patent 4,338,325 pursuant to 37 C.F.R. 1.785(b).
- (6) That the aforementioned election is not intended to waive any right of appeal should said election or alternative be denied a term extension.
- (7) That I believe that the Patent is subject to extension pursuant to 37 CFR 1.710.
- (8) That I believe that a 1346 day extension of the term of the Patent is justified under 35 U.S.C. 156 and applicable regulations.
- (9) That I believe the Patent meets the conditions for the extension of the term of a patent as set forth in 37 CFR 1.720.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of United States Patent 4,338,325 and any extensions thereof.



David J. Levy, Ph.D.
Reg. No. 27,655
Patent Counsel
Glaxo Wellcome Inc.
Five Moore Drive
Research Triangle Park, NC 27709

November 13, 1995
Date

EXHIBIT 1

**ASSIGNMENT OF U.S. PATENT 4,539,333
PARENT OF U.S. PATENT 4,883,812**

4539333

A S S I G N M E N T

WHEREAS I, salvador Moncada,
residing at 17 St. Davids Close, West Wickham, Kent, England.
have invented or discovered certain improvements in "Ethers"
hereinafter referred to as said invention and improvements for which I
have made a United States of America application for Letters Patent
filed 10th May 1977 Serial No. 795 524
and;

WHEREAS BURROUGHS WELLCOME CO., a corporation organized and
existing under and by virtue of the laws of the State of North Carolina
and having its principal place of business at Research Triangle Park,
North Carolina, is desirous of acquiring the whole right, title and
interest in and to said invention and improvements and application and
in and to any Letters Patent to be obtained therefor in the United
States, its territories and possessions; and

WHEREAS THE WELLCOME FOUNDATION LTD, a company incorporated
in England, whose registered office is at 183-193 Euston Road, London,
N.W.1, England, is desirous of acquiring the whole right, title and
interest in and to said invention and improvements and in and to any
applications for said invention and improvements and any Letters
Patent to be obtained therefor in all countries other than the United
States, its territories and possessions;

REEL 3479 FRAME 683

NOW, THEREFORE, to all whom it may concern, be it known that
I, Salvador Moncada
for good and valuable considerations unto me moving, the receipt
whereof is hereby acknowledged, have sold, assigned and transferred,
and by these presents do sell, assign and transfer my whole right,

title and interest in and to said invention and improvements to said BURROUGHS WELLCOME CO., throughout the United States of America, its territories and possessions, and in and to said application and any renewals, continuations, continuations-in-part, and any divisions thereof, and in and to any and all Letters Patent of the United States of America which may be granted on said invention and improvements and any reissue thereof including any priority rights under the International convention for the United States of America;

AND, MY whole right, title and interest in and to said invention and improvements to THE WELLCOME FOUNDATION LTD, in all other countries throughout the world, and in and to any applications in said other countries, and continuations-in-part, patents of addition, revalidation patents, patents of importation, registrations, and any renewals, extensions and divisions thereof, and in and to any and all Letters Patent of said all other countries which may be granted on said invention and improvements including any priority rights under the International Convention for all other countries of the Union;

REF 3479 FRAME 684

AND, I do hereby authorize and request the issue of any Letters Patent in the respective areas referred to, to said BURROUGHS WELLCOME CO. or THE WELLCOME FOUNDATION LTD, as assignees of my whole right, title and interest in and to the same for the sole use and behoof of the said assignees, their successors and assigns as their interests appear herein;

AND, I warrant that I have not knowingly conveyed to others any right in said invention, improvements, applications or patents or any license to use the same or to make, use or sell anything embodying or utilizing said invention and improvements and that I have good right to assign the same to BURROUGHS WELLCOME CO. or THE WELLCOME FOUNDATION LTD;

AND, I the undersigned Salvador Moncada
for the consideration aforesaid, do hereby agree that I or my executors
or legal representatives, will provide information and make, execute
and deliver any and all other instruments in writing, and any and all
further acts, application papers, affidavits, assignments and other
documents which may be necessary or desirable to more effectually
secure to and vest in said BURROUGHS WELLCOME CO. and THE WELLCOME
FOUNDATION LTD, their successors and assigns, the whole right, title
and interest in and to the said invention and improvements, applications,
Letters Patent, rights, title and interest, hereby sold, assigned and
conveyed, or intended so to be.

IN WITNESS WHEREOF, I have hereunto set my hand and affixed
my seal this eight day of November 1977.

Inventor's Signature

Salvador Moncada

SALVADOR MONCADA

GREAT BRITAIN
ENGLAND
COUNTY OF KENT

)
) ss.
)

REL 3479 FRAME 685

On this eight day of November 1977,
before me personally came SALVADOR MONCADA
to me known and known to me to be the individual described in and who
executed the foregoing assignment, and he thereupon acknowledged to me
that he executed same.



[Signature]
Notary Public of London, England

GREAT BRITAIN AND NORTHERN IRELAND
LONDON, ENGLAND
EMBASSY OF THE UNITED STATES OF AMERICA } ss.

I, Leslie A. Gerson Vice Consul of the United States of
America residing at London, England, duly commissioned and qualified,
do hereby certify that

EDWIN BRUCE WALKER

whose signature and official seal are respectively subscribed and
affixed to the annexed certificate, was on the date of the signing
thereof a Notary Public at London, England, duly authorized to
perform notarial acts, duly appointed and qualified, to whose
official acts faith and credit are due; that I have compared the
signature of said

EDWIN BRUCE WALKER

REF 3479 FRAME 686

on the annexed certificate with a specimen of his signature filed
in this Embassy; that I believe his signature to be genuine; that
I have compared the impression of the seal affixed thereto with a
specimen thereof filed in this Embassy, and that I believe the
impression of the seal upon the said original annexed certificate
to be genuine.

IN WITNESS WHEREOF I have hereunto
set my hand and affixed the seal of
the Consular Service of the United
States of America at London, England,
this tenth day of
November in the year of Our
Lord one thousand nine hundred and
seventy-seven.

Leslie Ann Gerson
Leslie A. Gerson
Vice Consul of the United States
of America at London, England.

Service Receipt No. GL477331

Tariff Item No. 49

Fee: ~~£2.50~~ £3.00

~~No fee prescribed~~

RECORDED
U.S. PATENT OFFICE

DEC - 7 1977

Sublette & Parker
ACTING COMMISSIONER OF
PATENTS & TRADEMARKS

LND/129
Feb. 77

EXHIBIT 2

CERTIFICATE OF MERGER
&
CERTIFICATE OF CHANGE OF NAME

STATE OF NORTH CAROLINA



Department of The
Secretary of State

To all whom these presents shall come, Greetings:

I, Rufus L. Edmisten, Secretary of State of the State of North Carolina, do hereby certify the following and hereto attached to be a true copy of

ARTICLES OF MERGER

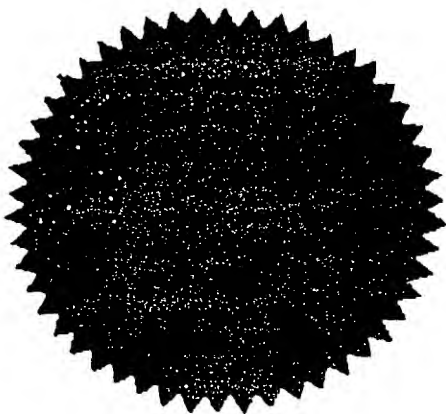
OF
GLAXO WELLCOME INC.
a North Carolina Corporation

INTO

BURROUGHS WELLCOME CO.
a North Carolina Corporation

the original of which was filed in this office on the 30th day of October, 1995.

IN WITNESS WHEREOF, I have hereunto set my hand and affixed my official seal at the City of Raleigh, this 30th day of October, 1995.



Rufus L. Edmisten

Secretary of State

STATE OF NORTH CAROLINA



Department of The
Secretary of State

To all whom these presents shall come, Greetings:

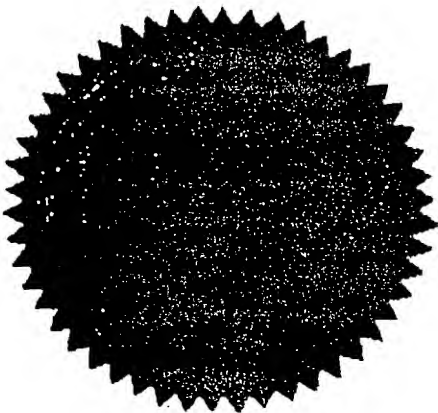
I, Rufus L. Edmisten, *Secretary of State of the State of North Carolina*, do hereby certify the following and hereto attached to be a true copy of

**ARTICLES OF AMENDMENT
OF**

**BURROUGHS WELLCOME CO.
name changed to:
GLAXO WELLCOME INC.**

the original of which was filed in this office on the 30th day of October, 1996.

IN WITNESS WHEREOF, I have hereunto set my hand and affixed my official seal at the City of Raleigh, this 30th day of October, 1996.



Rufus L. Edmisten

Secretary of State

.....

EXHIBIT 3

.....

MAINTENANCE FEE STATEMENT for U.S. PATENT 4,883,812

NO INFORMATION AVAILABLE IN THE AUTOMATED RECORDS
FOR PATENT NUMBER 4883812

AUTOMATED DATA COVERS ASSIGNMENTS RECORDED FROM AUGUST 5, 1980 TO DATE
FOR ASSIGNMENTS RECORDED BEFORE AUGUST 5, 1980, SEE CARD INDEX.

TO PROCESS ANOTHER QUERY PRESS THE 'P' AND 'XMIT' KEYS.

IF NO ADDITIONAL PATENT NUMBER QUERIES ARE DESIRED, PRESS 'XMIT'.

ENTER RESPONSE

10/23/95 13:07

Patent Maintenance Fees - Public Inquiry
Patent#: 4883812 Filed: 08/29/88 Issued: 11/28/89 Serial#: 07237987
Status: 8th Year Fee Window Opens: 11/28/96
Window Opens: 11/29/96 Surchg Due: 05/28/97
Fee Amt Due: \$ 1990 Surchg Amt Due: \$
Fee Code: 184 Surchg Code:
Title: TREATMENT OF HYPERTENSION USING PROSTACYCLIN

Address For Fee Purposes:
OLCOTT INTERNATIONAL & CO.
62 HACKENSACK PLANK ROAD
P.O. 3014
WEEHAWKEN, NJ. 07087

Most Recent Significant Events:
05/14/93 Payment of Maintenance Fee, 4th Year, Large Entity
09/28/90 Payer Number Assigned
Last Event On Maintenance History

EXHIBIT 4

FDA APPROVAL LETTER FOR
FLOLAN® (epoprostenol sodium) for Injection

#12

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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OFFICE OF PETITIONS
AND PATENTS

Re: Patent Term Extension for U.S. Patent 4,338,325

Commissioner of Patents and Trademarks
Box Patent Extension
Washington, DC 20231

Sir:

Transmitted herewith is an APPLICATION FOR EXTENSION OF PATENT TERM under 35 U.S.C. 156 with regard to U.S. Patent No. 4,883,812.

The Commissioner is hereby authorized to charge Deposit Account No. 02-4857 in the amount of \$1,030.00 to cover the application fee. The Commissioner is hereby authorized to charge any additional fees, which may be required, or credit overpayment to Account No. 02-4857. Triplicate copies of this letter are enclosed.

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this Application for Patent term Extension and the documents referred to therein are being deposited with the United States Postal Service on this date _____, 1995 in an envelope as "Express Mail Post Office to Addressee," Mailing Label Number _____ addressed to the: Commissioner of Patents and Trademarks Washington, D.C. 20231.

(Type or print name of person mailing paper)

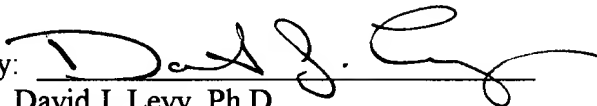
(Signature of person mailing paper)

Please address all communications relating to the enclosed APPLICATION FOR EXTENSION
OF PATENT TERM to:

David J. Levy, Ph.D.
Patent Counsel
Glaxo Wellcome Inc.
Intellectual Property Department
Five Moore Drive
Research Triangle Park, NC 27709

Telephone No. (919) 248-7656

Respectfully submitted,
Glaxo Wellcome Inc.

By: 
David J. Levy, Ph.D.
Reg. No. 27,655
Patent Counsel



DEPARTMENT OF HEALTH & HUMAN SERVICES

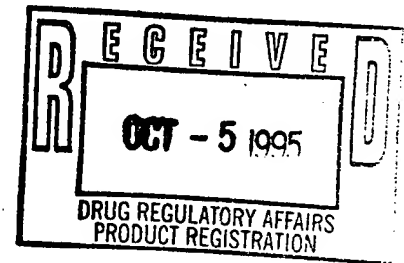
Public Health Service

Food and Drug Administration
Rockville MD 20857

NDA 20-444

SEP 20 1995

Burroughs Wellcome Company
Attention: Michael J. Dalton, Pharm.D.
3030 Cornwallis Road
Research Triangle Park, North Carolina 27709



Dear Dr. Dalton:

Please refer to your February 28, 1994 new drug application submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Flolan (epoprostenol sodium) for Injection.

We acknowledge receipt of your amendments dated April 28, May 22, June 6 and 9, and July 24, 1995.

This new drug application provides for the long-term intravenous treatment of primary pulmonary hypertension in NYHA Class III and Class IV adult patients.

We have completed the review of this application including the submitted draft labeling and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in the enclosed marked-up draft labeling. Accordingly, the application is approved effective on the date of this letter.

The final printed labeling (FPL) must be identical to the enclosed marked-up draft labeling. Marketing the product with FPL that is not identical to this draft labeling may render the product misbranded and an unapproved new drug.

Please submit sixteen copies of the FPL as soon as it is available, in no case more than 30 days after it is printed. Please individually mount ten of the copies on heavy weight paper or similar material. For administrative purposes this submission should be designated "FINAL PRINTED LABELING" for approved NDA 20-444. Approval of this labeling by FDA is not required before it is used.

Should additional information relating to the safety and effectiveness of the drug become available, revision of that labeling may be required.

We remind you of your Phase 4 commitments that, along with completion dates, are listed below:

1. The commitments agreed to in the April 27, 1995 telephone conversation between representatives of your firm and this Agency, and detailed in an Agency letter dated April 28, 1995 to submit to FDA additional information concerning the sterilization process.
2. The commitments agreed to in your April 10, 1995 submission, and confirmed in an Agency letter dated April 28, 1995, to submit to FDA, within 6 months of approval of the NDA, additional information regarding chemistry, manufacturing, and controls.

Protocols, data, and final reports should be submitted to your IND for this product and a copy of the cover letter sent to this NDA. Should an IND not be required to meet your Phase 4 commitments, please submit protocol, data, and final reports to this NDA as correspondences. For administrative purposes, all submissions, including labeling supplements, relating to these Phase 4 commitments must be clearly designated "Phase 4 Commitments."

In addition, please submit three copies of the introductory promotional material that you propose to use for this product. All proposed materials should be submitted in draft or mock-up form, not final print. Please submit one copy to the Division of Gastrointestinal and Coagulation Drug Products and two copies of both the promotional material and the package insert directly to:

Food and Drug Administration
Division of Drug Marketing, Advertising and
Communications, HFD-240
5600 Fishers Lane
Rockville, Maryland 20857

Validation of the regulatory methods has not been completed. At the present time, it is the policy of the Center not to withhold approval because the methods are being validated. Nevertheless, we expect your continued cooperation to resolve any problems that may be identified.

We remind you that you must comply with the requirements for an approved NDA set forth under 21 CFR 314.80 and 314.81.

If you have any questions, please contact:

Karen Oliver
Consumer Safety Officer
(301) 443-0487

Sincerely yours,

A handwritten signature in cursive script, appearing to read "Robert Temple".

Robert Temple, M.D.
Director
Office of Drug Evaluation 1
Center for Drug Evaluation and
Research

ENCLOSURE

EXHIBIT 5

**APPROVED PACKAGE INSERT FOR
FLOLAN® (epoprostenol sodium) for Injection
NDA 20-444**

1 **PACKAGE INSERT**

2 **FLOLAN® (epoprostenol sodium) for Injection**

3 **DESCRIPTION:** FLOLAN (epoprostenol sodium) for

4 Injection is a sterile sodium salt formulated for

5 intravenous administration. Each vial of FLOLAN

6 contains epoprostenol sodium equivalent to either

7 0.5 mg (500,000 ng) or 1.5 mg (1,500,000 ng)

8 epoprostenol, 3.76 mg glycine, 2.93 mg sodium

9 chloride, and 50 mg mannitol. Sodium hydroxide

10 may have been added to adjust pH.

11 Epoprostenol (PGI_2 , PGX, prostacyclin), a metabolite

12 of arachidonic acid, is a naturally occurring

13 prostaglandin with potent vasodilatory activity and

14 inhibitory activity of platelet aggregation.

15 Epoprostenol is (5Z,9,11,13E,15S)-6,9-epoxy-11,15-

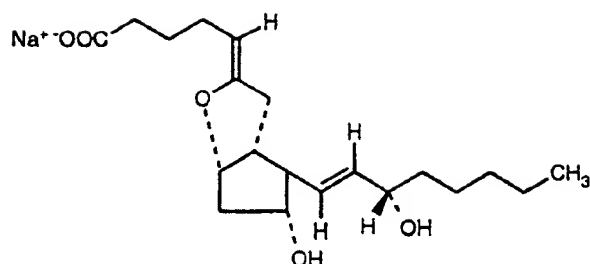
16 dihydroxyprosta-5,13-dien-1-oic acid.

17 Epoprostenol sodium has a molecular weight of

18 374.45 and a molecular formula of $\text{C}_{20}\text{H}_{31}\text{NaO}_5$. The

19 structural formula is:

20



21
 22 FLOLAN is a white to off-white powder that must be
 23 reconstituted with STERILE DILUENT for FLOLAN.
 24 STERILE DILUENT for FLOLAN is supplied in 50 mL
 25 glass vials containing 94 mg glycine, 73.5 mg sodium
 26 chloride, sodium hydroxide (added to adjust pH), and
 27 Water for Injection, USP.

28 The reconstituted solution of FLOLAN has a pH of
 29 10.2 to 10.8 and is increasingly unstable at a lower
 30 pH.

31 **CLINICAL PHARMACOLOGY:**

32 **General:** Epoprostenol has two major
 33 pharmacological actions: (1) direct vasodilation of
 34 pulmonary and systemic arterial vascular beds, and
 35 (2) inhibition of platelet aggregation. In animals, the
 36 vasodilatory effects reduce right and left ventricular
 37 afterload and increase cardiac output and stroke
 38 volume. The effect of epoprostenol on heart rate in
 39 animals varies with dose. At low doses, there is

40 vagally mediated bradycardia, but at higher doses,
41 epoprostenol causes reflex tachycardia in response
42 to direct vasodilation and hypotension. No major
43 effects on cardiac conduction have been observed.
44 Additional pharmacologic effects of epoprostenol in
45 animals include bronchodilation, inhibition of gastric
46 acid secretion, and decreased gastric emptying.

47 **Pharmacokinetics:** Epoprostenol is rapidly
48 hydrolyzed at neutral pH in blood and is also subject
49 to enzymatic degradation. Animal studies using
50 tritium-labelled epoprostenol have indicated a high
51 clearance (93 mL/min/kg), small volume of
52 distribution (357 mL/kg), and a short half-life
53 (2.7 minutes). During infusions in animals, steady-
54 state plasma concentrations of tritium-labelled
55 epoprostenol were reached within 15 minutes and
56 were proportional to infusion rates.

57 No available chemical assay is sufficiently sensitive
58 and specific to assess the in vivo human
59 pharmacokinetics of epoprostenol. The in vitro half-
60 life of epoprostenol in human blood at 37°C and pH
61 7.4 is approximately 6 minutes; the in vivo half-life of
62 epoprostenol in man is therefore expected to be no
63 greater than 6 minutes. The in vitro pharmacologic

64 half-life of epoprostenol in human plasma, based on
65 inhibition of platelet aggregation, was similar for
66 males (n = 954) and females (n = 1024).

67 Tritium-labelled epoprostenol has been administered
68 to humans in order to identify the metabolic products
69 of epoprostenol. Epoprostenol is metabolized to two
70 primary metabolites: 6-keto-PGF_{1α} (formed by
71 spontaneous degradation) and 6,15-diketo-13,14-
72 dihydro-PGF_{1α} (enzymatically formed), both of which
73 have pharmacological activity orders of magnitude
74 less than epoprostenol in animal test systems. The
75 recovery of radioactivity in urine and feces over a
76 one-week period was 82% and 4% of the
77 administered dose, respectively. Fourteen additional
78 minor metabolites have been isolated from urine,
79 indicating that epoprostenol is extensively
80 metabolized in man.

81 Clinical Trials: 

DELETE and REPLACE with:
CLINICAL TRIALS IN PRIMARY
PULMONARY HYPERTENSION (PPH)

82

83 **Hemodynamic Effects:** Acute intravenous infusions
84 of FLOLAN for up to 15 minutes in patients with
85 secondary and primary pulmonary hypertension
86 (PPH) produce dose-related increases in cardiac
87 index (CI) and stroke volume (SV), and dose-related

CORRECT TYPOGRAPHICAL ERROR:
(Insert space between run-on
words).

88 decreases in pulmonary vascular resistance (PVR),
89 total pulmonary resistance (TPR), and mean
90 systemic arterial pressure (SAPm). The effects of
91 FLOLAN on mean pulmonary artery pressure
92 (PAPm) in patients with PPH were variable and
93 minor.

94 Chronic continuous infusions of FLOLAN in patients
95 with PPH were studied in two prospective, open,
96 randomized trials of 8 and 12 weeks duration
97 comparing FLOLAN plus standard therapy to
98 standard therapy alone. Dosage of FLOLAN was
99 determined as described in DOSAGE AND
100 ADMINISTRATION and averaged 9.2 ng/kg/min at
101 the end of the 12-week trial. Standard therapy varied
102 among patients and included some or all of the
103 following: anticoagulants in essentially all patients;
104 oral vasodilators, diuretics, and digoxin in one-half to
105 two-thirds of patients; and supplemental oxygen in
106 about half the patients. Except for two New York
107 Heart Association (NYHA) functional Class II patients,
108 all patients were either functional Class III or Class IV.
109 As results were similar in the two studies, the pooled
110 results are described. Chronic hemodynamic effects
111 were generally similar to acute effects CI, SV, and
112 arterial oxygen saturation were increased, and PAPm,
113 right atrial pressure (RAP), TPR, and systemic
114 vascular resistance (SVR) were decreased in patients
115 who received FLOLAN chronically compared to those
116 who did not. Table 1 illustrates the treatment-related
117 hemodynamic changes in these patients after 8 or
118 12 weeks of treatment.

DELETE and REPLACE with:
at study end.

INSERT:
. (period at end of
sentence).

119

120

Table 1

121

Hemodynamics During Chronic Administration of FLOLAN

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126

Hemodynamic Parameter	Baseline		Mean change from baseline at end of treatment period ^a	
	FLOLAN (n = 52)	Standard Therapy (n = 54)	FLOLAN (n = 48)	Standard Therapy (n = 41)
CI (L/min/m ²)	2.0	2.0	0.3 ^{**}	-0.1
PAPm (mm Hg)	60	60	-5 ^{**}	1
PVR (Wood U)	16	17	-4 ^{**}	1
SAPm (mm Hg)	89	91	-4	-3
SV (mL/beat)	44	43	6 ^{**}	-1
TPR (Wood U)	20	21	-5 ^{**}	1

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^a At 8 weeks: FLOLAN n = 10; Standard Therapy n = 11.

At 12 weeks: FLOLAN n = 38; Standard Therapy n = 30.

^{**} Denotes statistically significant change between FLOLAN and Standard Therapy groups.

137

138

CI = cardiac index; PAPm = mean pulmonary arterial pressure; PVR = pulmonary vascular resistance;
SAPm = mean systemic arterial pressure; SV = stroke volume; TPR = total pulmonary resistance.

139 These hemodynamic improvements appeared to

140 persist when FLOLAN was administered for at least

141 36 months in an open, non-randomized study.

142 **Clinical Effects:** Exercise capacity, as measured by

143 the 6-minute walk test, improved significantly in

144 patients receiving continuous intravenous FLOLAN

145 plus standard therapy for 8 or 12 weeks compared to

146 those receiving standard therapy alone.

147 Improvements were apparent as early as the first

148 week of therapy, and in long-term follow-up appeared

DELETE

149 to be maintained beyond 2 years. Increases in

150 exercise capacity were accompanied by significant
151 improvement in dyspnea and fatigue, as measured by
152 the Congestive Heart Failure Questionnaire and the
153 Dyspnea Fatigue Index.

154 Survival was improved in NYHA functional Class III
155 and Class IV PPH patients treated with FLOLAN for
156 12 weeks in a multicenter, open, randomized, parallel
157 study. At the end of the treatment period, 8 of
158 40 patients receiving standard therapy alone died,
159 whereas none of the 41 patients receiving FLOLAN
160 died ($P = 0.003$).

161

162 **INDICATIONS AND USAGE:** FLOLAN is indicated ^{INSERT:}
~~in adults~~
163 for the long-term intravenous treatment of primary
164 pulmonary hypertension in NYHA Class III and
165 Class IV patients (see CLINICAL PHARMACOLOGY:
166 Clinical Trials).

167 **CONTRAINDICATIONS:** A large study evaluating
168 the effect of FLOLAN on survival in NYHA Class III
169 and IV patients with CHF due to severe left ventricular
170 systolic dysfunction was terminated after an interim
171 analysis of 471 patients revealed a higher mortality in
172 patients receiving FLOLAN plus standard therapy
173 than in those receiving standard therapy alone. The

174 chronic use of FLOLAN in patients with CHF due to
175 severe left ventricular systolic dysfunction is therefore
176 contraindicated

177 FLOLAN is also contraindicated in patients with
178 known hypersensitivity to the drug or to structurally-
179 related compounds.

180 **WARNINGS: FLOLAN must be reconstituted only**
181 **as directed using STERILE DILUENT for FLOLAN.**
182 **FLOLAN must not be reconstituted or mixed with**
183 **any other parenteral medications or solutions**
184 **prior to or during administration.**

185 **Abrupt Withdrawal:** Abrupt withdrawal (including
186 interruptions in drug delivery) or sudden large
187 reductions in dosage of FLOLAN may result in
188 symptoms associated with rebound pulmonary
189 hypertension, including dyspnea, dizziness, and
190 asthenia. In clinical trials, one Class III PPH patient's
191 death was judged attributable to the interruption of
192 FLOLAN. Abrupt withdrawal should be avoided.

193 **Pulmonary Edema:** Some patients with primary
194 pulmonary hypertension have developed pulmonary
195 edema during dose ranging, which may be

196 associated with pulmonary veno-occlusive disease.
197 FLOLAN should not be used chronically in patients
198 who develop pulmonary edema during dose ranging.

199 **Sepsis:** See ADVERSE REACTIONS: Adverse
200 Events Attributable to the Drug Delivery System.

201 **PRECAUTIONS:**

202 **General:** FLOLAN should be used only by clinicians
203 experienced in the diagnosis and treatment of PPH.
204 The diagnosis of PPH should be carefully established
205 by standard clinical tests to exclude secondary
206 causes of pulmonary hypertension.

207 FLOLAN is a potent pulmonary and systemic
208 vasodilator. Dose ranging with FLOLAN must be
209 performed in a setting with adequate personnel and
210 equipment for physiologic monitoring and emergency
211 care. Although dose ranging in clinical trials was
212 performed during right heart catheterization
213 employing a pulmonary artery catheter, the risk of
214 cardiac catheterization in patients with PPH should be
215 carefully weighed against the potential benefits.
216 During acute dose ranging, asymptomatic increases
217 in pulmonary artery pressure coincident with
218 increases in cardiac output occurred rarely. In such
219 cases, dose reduction should be considered, but

DELETE and REPLACE with:

Although dose ranging in clinical trials was performed during right heart catheterization employing a pulmonary artery catheter, in uncontrolled studies utilizing Flolan, the risk of cardiac catheterization in patients with PPH should be carefully weighed against the potential benefits.

220 such an increase does not imply that chronic
221 treatment is contraindicated.

222 During chronic use, FLOLAN is delivered
223 continuously on an ambulatory basis through a
224 permanent indwelling central venous catheter.

225 Unless contraindicated, anticoagulant therapy should
226 be administered to PPH patients receiving FLOLAN to
227 reduce the risk of pulmonary thromboembolism or
228 systemic embolism through a patent foramen ovale.

229 In order to reduce the risk of infection, aseptic
230 technique must be used in the reconstitution and
231 administration of FLOLAN as well as in routine
232 catheter care. Because FLOLAN is metabolized
233 rapidly, even brief interruptions in the delivery of
234 FLOLAN may result in symptoms associated with
235 rebound pulmonary hypertension including dyspnea,
236 dizziness, and asthenia. The decision to initiate
237 therapy with FLOLAN should be based upon the
238 understanding that there is a high likelihood that
239 intravenous therapy with FLOLAN will be needed for
240 prolonged periods, possibly years, and the patient's
241 ability to accept and care for a permanent
242 intravenous catheter and infusion pump should be
243 carefully considered.

244 Based on clinical trials, the acute hemodynamic

245 response to FLOLAN did not correlate well with
246 improvement in exercise tolerance or survival during
247 chronic use of FLOLAN. Dosage of FLOLAN during
248 chronic use should be adjusted at the first sign of
249 recurrence or worsening of symptoms attributable to
250 PPH or the occurrence of adverse events associated
251 with FLOLAN (see DOSAGE AND
252 ADMINISTRATION). Following dosage adjustments,
253 standing and supine blood pressure and heart rate
254 should be monitored closely for several hours.

255 **Information for Patients:** Patients receiving
256 FLOLAN should receive the following information:
257 **FLOLAN must be reconstituted only with STERILE**
258 **DILUENT for FLOLAN.** FLOLAN is infused
259 continuously through a permanent indwelling central
260 venous catheter via a small, portable infusion pump.
261 Thus, therapy with FLOLAN requires commitment by
262 the patient to drug reconstitution, drug administration,
263 and care of the permanent central venous catheter.
264 Sterile technique must be adhered to in preparing the
265 drug and in the care of the catheter, and even brief
266 interruptions in the delivery of FLOLAN may result in
267 rapid symptomatic deterioration. The decision to
268 receive FLOLAN for PPH should be based upon the
269 understanding that there is a high likelihood that

270 therapy with FLOLAN will be needed for prolonged
271 periods, possibly years, and the patient's ability to
272 accept and care for a permanent intravenous
273 catheter and infusion pump should be carefully
274 considered.

275 **Drug Interactions:** Additional reductions in blood
276 pressure may occur when FLOLAN is administered
277 with diuretics, antihypertensive agents, or other
278 vasodilators. When other anti-platelet agents or
279 anticoagulants are used concomitantly, there is the
280 potential for FLOLAN to increase the risk of bleeding.
281 However, patients receiving FLOLAN infusions in
282 clinical trials were maintained on anticoagulants
283 without evidence of increased bleeding. In clinical
284 trials, FLOLAN was used with digoxin, diuretics,
285 anticoagulants, oral vasodilators, and supplemental
286 oxygen.

287 **Carcinogenesis, Mutagenesis, Impairment of**
288 **Fertility:** Long-term studies in animals have not
289 been performed to evaluate carcinogenic potential. A
290 micronucleus test in rats revealed no evidence of
291 mutagenicity. The Ames test and DNA elution tests
292 were also negative, although the instability of
293 epoprostenol makes the significance of these tests
294 uncertain. Fertility was not impaired in rats given

295 FLOLAN by subcutaneous injection at doses up to

296 100 µg/kg/day, [600 g/m²/day, 2.5 times the

297 recommended human dose (4.6 ng/kg/min or 245.1

298 g/m²/day, i.v.) based on body surface area].

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299 **Pregnancy:** Pregnancy Category B. Reproductive

300 studies have been performed in pregnant rats and

301 rabbits at doses up to 100 g/kg/day (600 g/m²/day in

302 rats, 2.5 times the recommended human dose, and

303 1180 g/m²/day in rabbits, 4.8 times the

304 recommended human dose based on body surface

305 area) and have revealed no evidence of impaired

306 fertility or harm to the fetus due to FLOLAN. There

307 are, however, no adequate and well-controlled

308 studies in pregnant women. Because animal

309 reproduction studies are not always predictive of

310 human response, this drug should be used during

311 pregnancy only if clearly needed.

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INSERT:

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312 **Labor and Delivery:** The use of FLOLAN during

313 labor, vaginal delivery, or caesarean section has not

314 been adequately studied in humans.

315 **Nursing Mothers:** It is not known whether this drug

316 is excreted in human milk. Because many drugs are

317 excreted in human milk, caution should be exercised

318 when FLOLAN is administered to a nursing woman.

319 **Pediatric Use:** No adequate and well-controlled
320 studies have been performed in pediatric patients.
321 However, sixty-three pediatric patients less than
322 16 years of age with pulmonary hypertension have
323 received FLOLAN during acute dose ranging. The
324 mean maximum dose in patients less than 16 years
325 of age was significantly greater than the mean
326 maximum dose in adults during acute dose ranging
327 (25 ng/kg/min and 8.6 ng/kg/min, respectively). A
328 limited number of pediatric patients less than
329 16 years of age (n=5) with PPH in NYHA functional
330 Class III or Class IV have received FLOLAN
331 chronically in controlled clinical trials. In contrast to
332 acute dose ranging, mean chronic infusion rates were
333 only slightly higher for pediatric patients than for
334 adults, this difference did not reach statistical
335 significance. There were no important differences in
336 the adverse event profiles between adult and
337 pediatric patients.

DELETE and replace
with

*Pediatric Use: Safety and
effectiveness in pediatric
patients have not been
established.*

338 **Geriatric Use:** Clinical studies of FLOLAN did not
339 include sufficient numbers of patients aged 65 and
340 over to determine whether they respond differently
341 from younger patients. In general, dose selection for
342 an elderly patient should be cautious, reflecting the
343 greater frequency of decreased hepatic, renal, or

344 cardiac function and of concomitant disease or other
345 drug therapy.

346 **ADVERSE REACTIONS:** During clinical trials,
347 adverse events were classified as follows:
348 (1) adverse events during acute dose ranging,
349 (2) adverse events during chronic dosing, and
350 (3) adverse events associated with the drug delivery
351 system.

352 **Adverse Events During Acute Dose Ranging:**

353 During acute dose ranging, FLOLAN was
354 administered in 2 ng/kg/min increments until the
355 patients developed symptomatic intolerance. The
356 most common adverse events and the adverse
357 events that limited further increases in dose were
358 generally related to the major pharmacologic effect
359 of FLOLAN, vasodilation. The most common dose-
360 limiting adverse events were nausea and vomiting,
361 headache, hypotension, chest pain, dizziness, and
362 bradycardia. Table 2 lists the adverse events
363 reported during acute dose ranging in decreasing
364 order of frequency.

365

Table 2

366

Adverse Events During Acute Dose Ranging

367

368

Adverse Events Occurring in $\geq 1\%$ of Patients	FLOLAN (% of patients) (n = 391)
Flushing	58
Headache	49
Nausea/Vomiting	32
Hypotension	16
Anxiety, nervousness, agitation	11
Chest pain	11
Dizziness	8
Bradycardia	5
Abdominal pain	5
Musculoskeletal pain	3
Dyspnea	2
Back pain	2
Sweating	1
Dyspepsia	1
Hypesthesia/Paresthesia	1
Tachycardia	1

385

Adverse Events During Chronic Administration:

387 Interpretation of adverse events is complicated by the

388 clinical features of PPH, which are similar to some of

389 the pharmacologic effects of FLOLAN (e.g.,

390 dizziness, syncope). Adverse events probably related

391 to the underlying disease include dyspnea, fatigue,

392 chest pain, right ventricular failure, and pallor.

393 Several adverse events, on the other hand, can

394 clearly be attributed to FLOLAN. These include

395 headache, jaw pain, flushing, diarrhea, nausea and

396 vomiting, flu-like symptoms, and anxiety/nervousness.

397 In an effort to separate the adverse effects of the

398 drug from the adverse effects of the underlying

399 disease, table 3 lists adverse events that occurred at
 400 a rate at least 10% different in the two groups in
 401 controlled trials.

402 **Table 3**

403 **Adverse Events Regardless of Attribution Occurring with 10% Difference Between**
 404 **FLOLAN and Standard Therapy Alone**

INSERT:
 ≥

Adverse Event	FLOLAN (% of patients) (n = 52)	Standard therapy (% of patients) (n = 54)
Occurrence More Common with FLOLAN		
GENERAL		
Chills/Fever/Sepsis/Flu-like symptoms	25	11
CARDIOVASCULAR		
Tachycardia	35	24
Flushing	42	2
GASTROINTESTINAL		
Diarrhea	37	6
Nausea/Vomiting	67	48
MUSCULOSKELETAL		
Jaw Pain	54	0
Myalgia	44	31
Non-specific musculoskeletal pain	35	15
NEUROLOGICAL		
Anxiety/nervousness/tremor	21	9
Dizziness	83	70
Headache	83	33
Hypesthesia, Hyperesthesia, Paresthesia	12	2
Occurrence More Common With Standard Therapy		
CARDIOVASCULAR		
Heart failure	31	52
Syncope	13	24
Shock	0	13
RESPIRATORY		
Hypoxia	25	37

434 Thrombocytopenia has been reported during uncontrolled clinical trials in patients receiving FLOLAN.

435 Table 4 lists additional adverse events reported in PPH patients receiving FLOLAN plus standard

therapy or standard therapy alone during controlled clinical trials.

437

438

Table 4

439

Adverse Events Regardless of Attribution Occurring with <10% Difference Between FLOLAN
and Standard Therapy Alone

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GENERAL		
Asthenia	87 →	← 81
CARDIOVASCULAR		
Angina Pectoris	19	20
Arrhythmia	27	20
Bradycardia	15	9
Supraventricular tachycardia	8	0
Pallor	21	30
Cyanosis	31	39
Palpitation	63	61
Cerebrovascular accident	4	0
Hemorrhage	19	11
Hypotension	27	31
Myocardial ischemia	2	6
GASTROINTESTINAL		
Abdominal pain	27 →	← 31
Anorexia	25	30
Ascites	12 →	← 17
Constipation	6	2
METABOLIC		
Edema	60	63
Hypokalemia	6	4
Weight reduction	27	24
Weight gain	6	4
MUSCULOSKELETAL		
Arthralgia	6	0
Bone pain	0	4
Chest pain	67	65
NEUROLOGICAL		
Confusion	6	11
Convulsion	4	0
Depression	37	44
Insomnia	4	4
RESPIRATORY		
Cough increase	38	46
Dyspnea	90	85
Epistaxis	4	2
Pleural effusion	4	2
DERMATOLOGIC		
Pruritus	4	0
Rash	10	13

485	Sweating	15	20
486	SPECIAL SENSES		
487	Amblyopia	8	4
488	Vision abnormality	4	0

489 **Adverse Events Attributable to the Drug Delivery**

490 **System:** Chronic infusions of FLOLAN are delivered

491 using a small, portable infusion pump through an

492 indwelling central venous catheter. During controlled

493 trials of up to 12 weeks duration, 21% of patients

494 reported a local infection and 13% of patients

495 reported pain at the injection site. During long-term

496 follow-up, sepsis was reported at least once in 14%

497 of patients and occurred at a rate of 0.32 infections

498 per patient per year in patients treated with FLOLAN.

499 This rate was higher than reported in patients using

500 chronic indwelling central venous catheters to

501 administer parenteral nutrition, but lower than

502 reported in oncology patients using these catheters.

503 Malfunctions in the delivery system resulting in an

504 inadvertent bolus of or a reduction in FLOLAN were

505 associated with symptoms related to excess or

506 insufficient FLOLAN, respectively (see ADVERSE

507 REACTIONS: Adverse Events During Chronic

508 Administration and OVERDOSAGE):

509 **OVERDOSAGE:** Signs and symptoms of excessive

510 doses of FLOLAN during clinical trials are the

511 expected dose-limiting pharmacologic effects of

If the words "and OVERDOSAGE" are to be retained, the events described in the second paragraph of the OVERDOSAGE section should include its relationship to the delivery system.

512 FLOLAN, including flushing, headache, hypotension,
513 tachycardia, nausea, vomiting, and diarrhea.
514 Treatment will ordinarily require dose reduction of
515 FLOLAN.

516 One patient with secondary pulmonary hypertension
517 accidentally received 50 mL of an unspecified
518 concentration of FLOLAN. The patient vomited and
519 became unconscious with an initially unrecordable
520 blood pressure. FLOLAN was discontinued and the
521 patient regained consciousness within seconds. No
522 fatal events have been reported following overdosage
523 of FLOLAN.

524 ~~Single intravenous doses of FLOLAN at 10 and~~
525 ~~50 mg/kg, 2.5 million times a human dose of 20 g/kg~~
526 ~~for 15 minutes on a g/m² basis, were lethal to mice~~
527 ~~and rats, respectively. Symptoms of acute toxicity~~
528 ~~were hypoactivity, ataxia, loss of righting reflex, deep~~
529 ~~slow breathing, and hypothermia.~~

DELETE and REPLACE with:
Single intravenous doses
of FLOLAN at 10 and 50 mg/kg
(2703 and 27027 times the
recommended acute phase
human dose based on body
surface area) were lethal to
mice and rats, respectively.
Symptoms of acute toxicity
were hypoactivity, ataxia,
loss of righting reflex,
deep slow breathing, and
hypothermia.

530 **DOSAGE AND ADMINISTRATION:**

531 **Important Note: FLOLAN must be reconstituted**

532 **only with STERILE DILUENT for FLOLAN.**

533 Reconstituted solutions of FLOLAN must not be

534 diluted or administered with other parenteral solutions

535 or medications (see WARNINGS).

536 **Dosage:**

537 **Acute Dose Ranging:**

538 The initial chronic infusion rate of FLOLAN is

INSERT:

~~Adults:~~

539 determined by an acute dose-ranging procedure.

540 During controlled clinical trials, this procedure was

541 performed during cardiac catheterization (see

542 PRECAUTIONS), but in subsequent uncontrolled

543 clinical trials, acute doseranging was performed

544 without cardiac catheterization. In either case, the

545 infusion rate is initiated at 2 ng/kg/min and increased

546 in increments of 2 ng/kg/min every 15 minutes or

547 longer until dose-limiting pharmacologic effects are

548 elicited. The most common dose-limiting

549 pharmacologic effects during dose ranging are

550 nausea, vomiting, headache, hypotension, ~~chest pain,~~

INSERT:

but also include

(list ALL)

551 ~~dizziness, and bradycardia.~~ During acute dose

552 ranging in clinical trials, the mean maximum dose

553 which did not elicit dose-limiting pharmacologic

554 effects was 8.6 ± 0.3 ng/kg/min.

555 ***Continuous Chronic Infusion:***

556 ~~Chronic continuous infusion of FLOLAN should be~~
557 administered through a central venous catheter.
558 Temporary peripheral intravenous infusions may be
559 used until central access is established. Chronic
560 infusions of FLOLAN should be initiated at
561 4 ng/kg/min less than the maximum-tolerated infusion
562 rate determined during acute dose ranging. If the
563 maximum-tolerated infusion rate is less than
564 5 ng/kg/min, the chronic infusion should be started at
565 one-half the maximum-tolerated infusion rate. During
566 clinical trials, the mean initial chronic infusion rate
567 was 5 ng/kg/min.

~~INSERT:
Adults:~~

568 **Dosage Adjustments:** Changes in the chronic
569 infusion rate should be based on persistence,
570 recurrence, or worsening of the patient's symptoms of
571 PPH and the occurrence of adverse events due to
572 excessive doses of FLOLAN. In general, increases in
573 dose from the initial chronic dose should be
574 expected. In the controlled 12-week trial, for
575 example, the dose increased from a mean starting
576 dose of 5.2 g/kg/min (4 g/kg/min less than the new
577 tolerated dose) to 9.2 g/kg/min by the end of
578 week 12, just 1.6 g/kg/min less than the mean non-
579 tolerated dose.

INSERT:

μ

INSERT:

μ

INSERT:

μ

580 Increments in dose should be considered if
581 symptoms of PPH persist or recur after improving.
582 The infusion should be increased by 1 to 2 ng/kg/min
583 increments at intervals sufficient to allow assessment
584 of clinical response; these intervals should be at least
585 15 minutes. Following establishment of a new
586 chronic infusion rate, the patient should be observed
587 and standing and supine blood pressure and heart
588 rate monitored for several hours to ensure that the
589 new dose is tolerated.

INSERT:

, (comma)

590 During chronic infusion, the occurrence of dose-
591 related pharmacological events similar to those

592 observed during acute dose ranging may necessitate
593 a decrease in infusion rate, but the adverse event
594 may occasionally resolve without dosage adjustment.
595 Dosage decreases should be made gradually in
596 2 ng/kg/min decrements every 15 minutes or longer
597 until the dose-limiting effects resolve. Abrupt
598 withdrawal of FLOLAN or sudden large reductions in
599 infusion rates should be avoided. Except in life-
600 threatening situations (e.g., unconsciousness,
601 collapse, etc.), infusion rates of FLOLAN should be
602 adjusted only under the direction of a physician.

603 In patients receiving lung transplants, doses of
604 FLOLAN were tapered after the initiation of
605 cardiopulmonary bypass.

606 **Administration:** FLOLAN is administered by
607 continuous intravenous infusion via a central venous
608 catheter using an ambulatory infusion pump. During
609 dose-ranging, FLOLAN may be administered
610 peripherally.

611 The ambulatory infusion pump used to administer
612 FLOLAN should: (1) be small and lightweight, (2) be
613 able to adjust infusion rates in 2 ng/kg/min
614 increments, (3) have occlusion, end of infusion, and
615 low battery alarms, (4) be accurate to $\pm 6\%$ of the

616 programmed rate, and (5) be positive pressure driven
617 (continuous or pulsatile) with intervals between
618 pulses not exceeding 3 minutes at infusion rates used
619 to deliver FLOLAN. The reservoir should be made of
620 polyvinyl chloride, polypropylene, or glass. Infusion
621 pumps used in clinical trials were the CADD-1
622 HFX 5100 (Pharmacia Deltec), Walk-Med 410 C
623 (Medfusion, Inc.), and the Auto Syringe AS2F (Baxter
624 Health Care).

625 To avoid potential interruptions in drug delivery, the
626 patient should have access to a backup infusion
627 pump and intravenous infusion sets. A multi-lumen
628 catheter should be considered if other intravenous
629 therapies are routinely administered.

630 To facilitate extended use at temperatures exceeding
631 25°C (77°F), a cold pouch with frozen gel packs was
632 used in clinical trials (see DOSAGE AND
633 ADMINISTRATION: Storage and Stability). The cold
634 pouches and gel packs used in clinical trials were
635 obtained from Palco Labs, Palo Alto, California.

INSERT:
ambient

← ADD:
Any cold pouch used
must be capable of
maintaining the
temperature of
reconstituted FLOLAN
between 2° and 8°C
for 12 hours.

636 **Reconstitution: FLOLAN is only stable when**
637 **reconstituted with STERILE DILUENT for FLOLAN.**
638 **FLOLAN must not be reconstituted or mixed with**
639 **any other parenteral medications or solutions**
640 **prior to or during administration.**

641 ~~Parenteral drug products should be inspected visually~~
642 ~~for particulate matter and discoloration prior to~~
643 ~~administration whenever solution and container~~
644 ~~permit. If either occurs, FLOLAN should not be~~
645 ~~administered.~~

DELETE

646 A concentration for the solution of FLOLAN for acute
647 dose ranging or chronic therapy should be selected
648 which is compatible with the infusion pump being used
649 with respect to minimum and maximum flow rates,
650 reservoir capacity, and the infusion pump criteria listed
651 above. FLOLAN, when administered chronically,
652 should be prepared in a drug delivery reservoir
653 appropriate for the infusion pump with a total reservoir
654 volume of at least 100 mL. FLOLAN should be
655 prepared using 2 vials of STERILE DILUENT for FLOLAN
656 for use during a 24-hour period. Table 5 gives
657 directions for preparing several different
658 concentrations of FLOLAN:

659

660

Table 5

To make 100 mL of solution with final Concentration (ng/mL) of:	Directions:
3,000 ng/mL	Dissolve contents of one 0.5 mg vial with 5 mL of STERILE DILUENT for FLOLAN. Withdraw 3 mL and add to sufficient STERILE DILUENT for FLOLAN to make a total of 100 mL.
5,000 ng/mL	Dissolve contents of one 0.5 mg vial with 5 mL of STERILE DILUENT for FLOLAN. Withdraw entire vial contents and add sufficient STERILE DILUENT for FLOLAN to make a total of 100 mL.
10,000 ng/mL	Dissolve contents of two 0.5 mg vials each with 5 mL of STERILE DILUENT for FLOLAN. Withdraw entire vial contents and add sufficient STERILE DILUENT for FLOLAN to make a total of 100 mL.
15,000 ng/mL	Dissolve contents of one 1.5 mg vial with 5 mL of STERILE DILUENT for FLOLAN. Withdraw entire vial contents and add sufficient STERILE DILUENT for FLOLAN to make a total of 100 mL.

Higher concentrations may be required for patients who receive

FLOLAN long-term.

More than one solution strength may be required to accommodate the range of infusions anticipated during acute dose-ranging. Generally, 3,000 ng/mL and 10,000 ng/mL are satisfactory concentrations to deliver between 2 to 16 ng/kg/min in adults. Infusion rates may be calculated using the following formula:

$$\text{Infusion Rate (mL/hr)} = \frac{[\text{Dose (ng/kg/min)} \times \text{Weight (kg)} \times 60 \text{ min/hr}]}{\text{Final Concentration (ng/mL)}}$$

Tables 6 through 9 provide infusion delivery rates for doses up to 16 ng/kg/min based upon patient weight,

682 drug delivery rate, and concentration of the solution of
 683 FLOLAN to be used. These tables may be used to
 684 select the most appropriate concentration of FLOLAN
 685 that will result in an infusion rate between the
 686 minimum and maximum flow rates of the infusion
 687 pump and which will allow the desired duration of
 688 infusion from a given reservoir volume. Higher
 689 infusion rates, and therefore, more concentrated
 690 solutions may be necessary with long-term
 691 administration of FLOLAN.

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Table 6

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Infusion Rates for FLOLAN at a Concentration of 3,000 ng/mL								
Patient Weight (kg)	Dose or Drug Delivery Rate (ng/kg/min)							
	2	4	6	8	10	12	14	16
Infusion Delivery Rate (mL/hr)								
10	—	—	1.2	1.6	2.0	2.4	2.8	3.2
20	—	1.6	2.4	3.2	4.0	4.8	5.6	6.4
30	1.2	2.4	3.6	4.8	6.0	7.2	8.4	9.6
40	1.6	3.2	4.8	6.4	8.0	9.6	11.2	12.8
50	2.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0
60	2.4	4.8	7.2	9.6	12.0	14.4	16.8	19.2
70	2.8	5.6	8.4	11.2	14.0	16.8	19.6	22.4
80	3.2	6.4	9.6	12.8	16.0	19.2	22.4	25.6
90	3.6	7.2	10.8	14.4	18.0	21.6	25.2	28.8
100	4.0	8.0	12.0	16.0	20.0	24.0	28.0	32.0

Table 7

Infusion Rates for FLOLAN at a Concentration of 5,000 ng/mL								
Patient Weight (kg)	Dose or Drug Delivery Rate (ng/kg/min)							
	2	4	6	8	10	12	14	16
Infusion Delivery Rate (mL/hr)								
10	—	—	—	1.0	1.2	1.4	1.7	1.9
20	—	1.0	1.4	1.9	2.4	2.9	3.4	3.8
30	—	1.4	2.2	2.9	3.6	4.3	5.0	5.8
40	1.0	1.9	2.9	3.8	4.8	5.8	6.7	7.7
50	1.2	2.4	3.6	4.8	6.0	7.2	8.4	9.6
60	1.4	2.9	4.3	5.8	7.2	8.6	10.1	11.5
70	1.7	3.4	5.0	6.7	8.4	10.1	11.8	13.4
80	1.9	3.8	5.8	7.7	9.6	11.5	13.4	15.4
90	2.2	4.3	6.5	8.6	10.8	13.0	15.1	17.3
100	2.4	4.8	7.2	9.6	12.0	14.4	16.8	19.2

Table 8

Infusion Rates for FLOLAN at a Concentration of 10,000 ng/mL							
Patient Weight (kg)	Dose or Drug Delivery Rate (ng/kg/min)						
	4	6	8	10	12	14	16
Infusion Delivery Rate (mL/hr)							
20	—	—	1.0	1.2	1.4	1.7	1.9
30	—	1.1	1.4	1.8	2.2	2.5	2.9
40	1.0	1.4	1.9	2.4	2.9	3.4	3.8
50	1.2	1.8	2.4	3.0	3.6	4.2	4.8
60	1.4	2.2	2.9	3.6	4.3	5.0	5.8
70	1.7	2.5	3.4	4.2	5.0	5.9	6.7
80	1.9	2.9	3.8	4.8	5.8	6.7	7.7
90	2.2	3.2	4.3	5.4	6.5	7.6	8.6
100	2.4	3.6	4.8	6.0	7.2	8.4	9.6

Table 9

Infusion Rates for FLOLAN at a Concentration of 15,000 ng/mL							
Patient	Dose or Drug Delivery Rate (ng/kg/min)						
Weight (kg)	4	6	8	10	12	14	16
Infusion Delivery Rate (mL/hr)							
30	—	—	1.0	1.2	1.4	1.7	1.9
40	—	1.0	1.3	1.6	1.9	2.2	2.6
50	—	1.2	1.6	2.0	2.4	2.8	3.2
60	1.0	1.4	1.9	2.4	2.9	3.4	3.8
70	1.1	1.7	2.2	2.8	3.4	3.9	4.5
80	1.3	1.9	2.6	3.2	3.8	4.5	5.1
90	1.4	2.2	2.9	3.6	4.3	5.0	5.8
100	1.6	2.4	3.2	4.0	4.8	5.6	6.4

768 **Storage and Stability:** Unopened vials of FLOLAN
 769 are stable until the date indicated on the package
 770 when stored at 15° to 25°C (59° to 77°F) and
 771 protected from light in the carton. Unopened vials of
 772 STERILE DILUENT for FLOLAN are stable until the
 773 date indicated on the package when stored at 15° to
 774 25°C (59° to 77°F).

775 Prior to use, reconstituted solutions of FLOLAN must
776 be protected from light and must be refrigerated at
777 2° to 8°C (36° to 46°F) if not used immediately. Do
778 not freeze reconstituted solutions of FLOLAN.
779 Discard any reconstituted solution that has been
780 frozen. ←

ADD:

Discard any reconstituted
solution if it has been
refrigerated for more than
48 hours.

781 During use, a single reservoir of reconstituted solution
782 of FLOLAN can be administered at room temperature
783 for a total duration of 8 hours, or it can be
784 administered up to 24 hours with the use of two
785 frozen 6-oz gel packs in a cold pouch. When stored
786 or in use, reconstituted FLOLAN must be insulated
787 from temperatures greater than 25°C (77°F) and less
788 than 0°C (32°F), and must not be exposed to direct
789 sunlight.

INSERT:

used with a cold pouch and

790 **Use at Room Temperature:** Prior to use at room
791 temperature, 15° to 25°C (59° to 77°F), reconstituted
792 solutions of FLOLAN may be stored refrigerated at 2°
793 to 8°C (36° to 46°F) for no longer than 40 hours.
794 When administered at room temperature,
795 reconstituted solutions may be used for no longer
796 than 8 hours. This 48-hour period allows the patient
797 to reconstitute a 2-day supply (200 mL) of FLOLAN.
798 Each 100 mL daily supply may be divided into three

799 equal portions. Two of the portions are stored
800 refrigerated at 2° to 8°C (36° to 46°F) until they are
801 used.

802 **Use with a Cold Pouch:** Prior to infusion with the
803 use of a cold pouch, solutions may be stored
804 refrigerated at 2° to 8°C (36° to 46°F) for up to
805 24 hours. When a cold pouch is employed during the
806 infusion, reconstituted solutions of FLOLAN may be
807 used for no longer than 24 hours. The gel packs
808 should be changed every 12 hours.

ADD:

Reconstituted solutions may be kept at 2°C to 8°C (36° to 46°F), either in refrigerated storage or in a cold pouch or a combination of the two, for no more than 48 hours.

809 **HOW SUPPLIED:** FLOLAN for Injection is supplied
810 as a sterile freeze-dried powder in 17 mL flint glass
811 vials with gray butyl rubber closures, individually
812 packaged in a carton.

813 17 mL vial containing epoprostenol sodium equivalent
814 to 0.5 mg (500,000 ng), carton of 1, (NDC 0081-
815 0460-01).

ADD NEW PARAGRAPH:

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit. If either occurs, reconstituted FLOLAN should not be administered.

816 17 mL vial containing epoprostenol sodium equivalent
817 to 1.5 mg (1,500,000 ng), carton of 1, (NDC 0081-
818 0464-01).

819 Store the vials of FLOLAN at 15° to 25°C (59° to
820 77°F). Protect from light.

821 The STERILE DILUENT for FLOLAN is supplied in 50
822 mL flint glass vials with fluororesin faced butyl rubber
823 closures.

824 50 mL vial of STERILE DILUENT for FLOLAN, tray of 4
825 (NDC 0081-0462-01).

826 Store the vials of STERILE DILUENT for FLOLAN at
827 15° to 25°C (59° to 77°F). DO NOT FREEZE.

828 **Caution:** Federal law prohibits dispensing without
829 prescription.

830

831 U.S. Patent Nos. 4335139, 4539333, and 4883812 (Use Patent)
832 Licensed Under U.S. Patent No. 4338325

833
834

835 Manufactured by
836 THE WELLCOME FOUNDATION LTD.
837 London, England NW1 2BP for
838 BURROUGHS WELLCOME CO.
839 Research Triangle Park, NC 27709

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842 Printed in U.S.A. (Date of Issue) (Item No.)
843

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845

EXHIBIT 6

U.S. PATENT 4,883,812

United States Patent [19]

Moncada

[11] Patent Number: 4,883,812

[45] Date of Patent: * Nov. 28, 1989

[54] TREATMENT OF HYPERTENSION USING PROSTACYCLIN

[75] Inventor: Salvador Moncada, West Wickham, England

[73] Assignee: Burroughs Wellcome Co., Research Triangle Park, N.C.

[*] Notice: The portion of the term of this patent subsequent to Sep. 3, 2002 has been disclaimed.

[21] Appl. No.: 237,987

[22] Filed: Aug. 29, 1988

Related U.S. Application Data

[63] Continuation of Ser. No. 712,788, Mar. 18, 1985, which is a continuation of Ser. No. 795,524, May 10, 1977, Pat. No. 4,539,333.

[30] Foreign Application Priority Data

Aug. 17, 1976 [GB] United Kingdom 34151

Sep. 3, 1976 [GB] United Kingdom 36547

[51] Int. Cl.⁴ A61K 31/557; A61K 31/34

[52] U.S. Cl. 514/469

[58] Field of Search 514/469

[56] References Cited

U.S. PATENT DOCUMENTS

4,338,325 7/1982 Johnson et al. 514/469

4,430,340 7/1984 Cho 514/469

4,499,293 2/1985 Johnson et al. 549/465

4,539,333 9/1985 Moncada 514/469

Primary Examiner—Mary C. Lee

Assistant Examiner—Bernard I. Dentz

Attorney, Agent, or Firm—Donald Brown

[57] ABSTRACT

Prostacyclin, its salts biosynthesis and synthesis thereof, pharmaceutical formulations containing them, and their use in medicine.

4 Claims, No Drawings

TREATMENT OF HYPERTENSION USING PROSTACYCLIN

This is a continuation of co-pending application Ser. No. 712,788 filed on Mar. 18, 1985 which is a continuation of Ser. No. 795,524 filed on May 10, 1977 U.S. Pat. No. 4,539,333.

This invention relates to the extraction, isolation and synthesis of a prostaglandin derivative, its use as a chemical intermediate, formulations containing it, and its use in medicine.

Prostaglandin endoperoxides (PGG₂ and PGH₂) are generated from arachidonic acid by a membrane-bound cyclo-oxygenase enzyme system, described by Hamberg and Samuelsson (Biochem. Biophys. Acta. 326, 448-461, 1974) and are subsequently transformed to PGF_{2α}, PGE₂, PGD₂, or Thromboxane A₂. Thromboxane A₂ shares with the prostaglandin endoperoxides the important biological properties of contracting strips of rabbit aorta and aggregating blood platelets.

The Applicants have now found that microsomes derived from a variety of mammalian tissues catalyze the enzymic transformation of the prostaglandin endoperoxides to a prostaglandin derivative (hereinafter referred to as Prostacyclin which does not contact strips of rabbit aorta, relaxes strips of rabbit coeliac mesenteric and coronary arteries, has a potent anti-aggregatory action on blood platelets, is a strong vasodilator in whole animals and has other properties described hereinafter.

Microsomes derived from rabbit or pig blood vessels such as veins and arteries, and rat stomach fundus produce about 80-90% conversion of the prostaglandin endoperoxides. Microsomes derived from rabbit lung tissue and rat pyloric tissue produce 25% conversion of the prostaglandin endoperoxides, whereas those derived from rat kidney, brain, spleen, liver, heart and seminal vesicle tissues produce 5% or less conversion of the prostaglandin endoperoxides.

Prostacyclin is unstable at room temperature in aqueous medium, having a half-life of approximately 10 mins., but its anti-aggregatory activity can be preserved for several days by dissolving the substance in aqueous alkali or in dry acetone and storing at -20° C. On average prostacyclin is 10-40 times more potent as an anti-aggregatory agent than PGE₁ and 5-20 times more potent than PGD₂; itself a potent inhibitor of platelet aggregation. Prostacyclin also interrupts and reverses the presence of platelet aggregation.

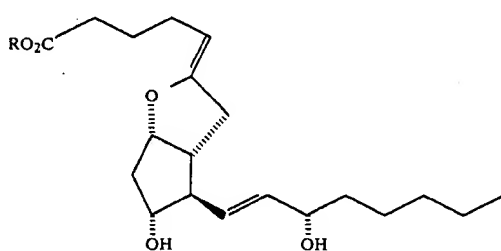
Prostacyclin may be prepared biosynthetically by incubating PGG₂ or PGH₂ with aortic microsomes in a suitable buffer solution such as Tris buffer, for approximately 2 minutes at a temperature in the region of 22° C. Conversion of the prostaglandin endoperoxides is approximately 85%.

Extraction of Prostacyclin is achieved by the addition of cold (0° C.) dry diethyl ether to the incubation mixture. The addition of cold ether stops the enzyme reaction and Prostacyclin enters the ether phase which may be separated from the aqueous phase. Evaporation of the ether by standard techniques such as bubbling nitrogen through the solution results in the Prostacyclin being left as a residue which may subsequently be resuspended in an aqueous solution for further examination or dissolved in anhydrous acetone and stored at a temperature in the region of -20° C. for future use.

The aortic microsomes employed in the incubation mixture may be extracted from pig or rabbit aortas. Aortas may be frozen solid preferably by dropping them in liquid nitrogen, and then crushed to form a powder which is resuspended in a suitable buffer solution and subsequently homogenized. The homogenate may then undergo sequential centrifugation so as to isolate the microsomal fraction which may be resuspended in deionized water and lyophilized. The incubation mixture was shown to have an immediate anti-aggregatory effect by monitoring the aggregation of human blood platelets in a Born aggregometer.

Prostacyclin may be prepared using other tissues identified above in substantially a similar manner. Prostacyclin formed in such an incubation mixture appears to be different from the other products of PG endoperoxides so far described. Its biological properties on the isolated tissues, its instability and its potent anti-aggregatory activity show that Prostacyclin is not being PGE₂ or PGF_{2α}. The presence of prostaglandin D₂ isomerase in homogenates of several tissues has been described. As prostacyclin is unstable and is a more potent anti-aggregatory agent than PGD₂, it cannot be regarded as PGD₂. Furthermore, PGD₂ isomerase is present in the 100,000 g. supernatant, a fraction that did not produce Prostacyclin from PG endoperoxides. Moreover, PGD₂ isomerase needs glutathione as a co-factor and the incubations were carried out in the absence of cofactors. PGE₂, PGF_{2α} and PGD₂ were not substrates for aortic microsomes and therefore 15-keto PGs and other products of prostaglandin catabolism could not be considered as Prostacyclin. Prostacyclin is also unlikely to be a known 15-hydroperoxy PG, firstly because 15-hydroperoxy PGE₂ has a contractile activity on rabbit aortic strip and secondly the product(s) of the spontaneous decay of Prostacyclin when bioassayed did not behave like PGE₂, PGF_{2α}, or PGD₂. As Prostacyclin has an anti-aggregatory activity it cannot be identical to Thromboxane A₂ or B₂ as they are pro-aggregatory substances.

Further studies have shown that Prostacyclin has the chemical structure shown in formula (I) (R is hydrogen) (see Johnson et al., Prostaglandins, 12/6, 915-928, 1976).

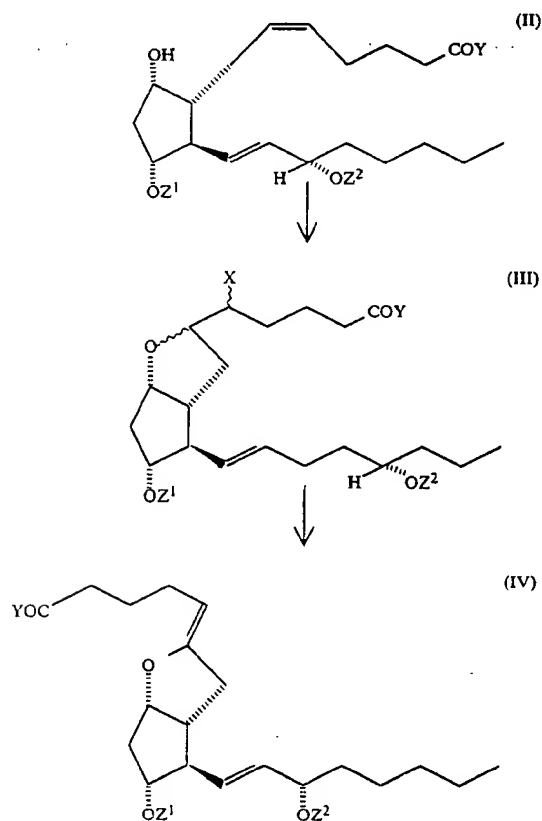


(I)

By the present invention we provide the compounds of formula (I) wherein R is hydrogen or a pharmacologically acceptable cation (hereinafter referred to as Prostacyclin and salts thereof). Salts of Prostacyclin include alkali metal salts, alkaline earth metal salts and salts of organic bases.

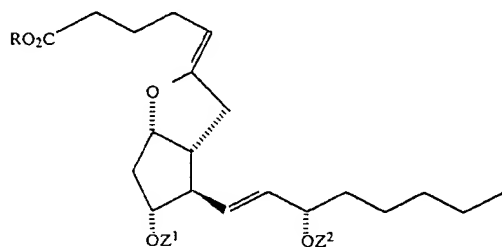
Salts of Prostacyclin may be synthesised from a compound of formula (II) wherein either or both of Z¹ and Z² is hydrogen or a blocking group such as acyl, or trialkylsilyl (for example trimethylsilyl). Oxidative attack by iodine or potassium triiodide in the presence of

a metal bicarbonate at the 5,6-double bond of a compound of formula (II) with simultaneous or subsequent cyclisation involving the 9-hydroxy group produces a compound of formula (III). Upon treatment with a suitable base such as an organic base or a metal alkoxide, a compound of formula (III) may then be dehydrohalogenated, resulting in the introduction of a 5,6-double bond. This reaction sequence is illustrated in the following reaction scheme:



wherein Y is OH, NHR¹ or OR¹, R¹ being alkyl of 1 to 4 carbon atoms or a cation; X is iodo or bromo; and Z¹ and Z² are as defined above.

Included in compounds of formula (IV) are compounds of formula



wherein R is hydrogen or a pharmacologically acceptable cation and each of Z¹ and Z² is hydrogen or a blocking group.

When Z¹ and/or Z² in formulae (II), (III) and (IV) are blocking groups, the resulting blocked derivatives of the compounds of formula (I) may be converted to

the corresponding compounds of formula (I) by methods known in the art, for example base hydrolysis.

Prostacyclin salts may be prepared by treating an ester thereof (formula (I) : R is alkyl) for example the methyl ester, with a strong base such as sodium hydroxide in a suitable solvent and lyophilising the resulting reaction mixture. Prostacyclin itself may be conveniently prepared by base hydrolysis of its corresponding esters or amides in the presence of an equivalent amount of a caustic alkali in an aqueous alcohol or an aqueous tetrahydrofuran medium, and extracted into an organic solvent at low temperature.

Prostacyclin and its salts are useful as intermediates in the synthesis of prostaglandin analogues, and exhibit a potent anti-aggregatory action on blood platelets, and therefore have a particular utility in the treatment and/or prophylaxis of mammals as anti-thrombotic agents.

They are also useful in mammals, including man, to reduce and control excessive gastric secretion, thereby reducing or avoiding gastrointestinal ulcer formation, and accelerating the healing of such ulcers and lesions already present in the gastrointestinal tract.

Prostacyclin and its salts further exhibit vasodilatory action on blood vessels and therefore have a particular utility as anti-hypertensives for the treatment of high blood pressure in mammals, including man. Use as an antihypertensive may be accomplished by administering a pharmaceutical solution containing the anion of (5Z)-5,6-Didehydro-9-deoxy-6,9-epoxy prostaglandin F_{1α}. Platelets can be assimilated into the vascular endothelium or even incorporated into endothelial cells. Biochemical co-operation between platelets and vascular endothelium in the generation of Prostacyclin contributes to the repair of vascular endothelium, and Prostacyclin and its salts have a further utility in the promotion of wound healing in mammals, including man.

Prostacyclin and its salts may be used whenever it is desired to inhibit platelet aggregation, to reduce the adhesive character of platelets, and to treat or prevent the formation of thrombi in mammals, including man. For example, they may be used in the treatment and prevention of myocardial infarcts, in the treatment of peripheral vascular disease to treat and prevent post-operative thrombosis, to promote patency of vascular grafts following surgery, and to treat complications of arteriosclerosis and conditions such as atherosclerosis, blood clotting defects due to lipemia, and other clinical conditions in which the underlying etiology is associated with lipid imbalance or hyperlipidemia.

They may also be used as additives to blood, blood products, blood substitutes, and other fluids which are used in artificial extra-corporeal circulation and perfusion of isolated body portions, e.g., limbs and organs, whether attached to the original body, detached and being preserved or prepared for transplant, or attached to a new body. During these circulations and perfusions, aggregated platelets tend to block the blood vessels and portions of the circulation apparatus. This blocking is avoided by the presence of Prostacyclin. For this purpose, Prostacyclin or its salts may be added gradually or in single or multiple portions to the circulating blood, to the blood of the donor animal, to the perfused body portion, attached or detached to the recipient, or to two or all of those at a total steady state dose of 0.001 to 10 mg., per liter of circulating fluid. It is especially useful to use Prostacyclin in laboratory animals, e.g. cats, dogs, rabbits, monkeys and rats, for

these purposes in order to develop new methods and techniques for organ and limb transplants.

The amount of prostacyclin or a salt thereof required (hereinafter referred to as the active ingredient) for therapeutic effect will vary with the route of administration. In general a suitable dose for a mammal will lie in the range of 0.01 to 200 mg. per kilogram body-weight, conveniently 0.01 to 10 mg per kilogram.

While it is possible for the active ingredient to be administered as the raw chemical it is preferable to present it as a pharmaceutical formulation. Such formulations are preferably non-aqueous and non-hydroxylic in nature, but alkaline aqueous solutions may be used. Unit doses of a formulation contain between 0.5 mg. and 1.5 g of the active ingredient.

Such formulations, both for veterinary and for human medical use, of the present invention comprise the active ingredient, as above defined, together with one or more acceptable carriers therefor and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The formulations include those suitable for parenteral (including subcutaneous, intramuscular and intravenous) administration which must of course be sterile.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired formulation.

According to the present invention there are therefore provided:

- (a) the compounds of formula (I) and derivatives thereof blocked in the 11- and 15- positions;
- (b) the preparation of Prostacyclin comprising:
 - (i) incubation of microsomes of fresh mammalian tissue with a prostaglandin endoperoxide, and the extraction of prostacyclin from the incubation mixture into an organic solvent; or
 - (ii) its synthesis as hereinbefore described;
- (c) Prostacyclin when obtained by the process described in paragraph (b);
- (d) a pharmaceutical formulation containing prostacyclin;
- (e) the preparation of such pharmaceutical formulations;
- (f) method for the treatment or prophylaxis of thrombosis in a mammal or mammalian tissues, including man, comprising the administration of a non-toxic, prophylactic anti-thrombotic amount of a compound of formula (I).
- (g) method for inducing vasodilation in a mammal, including man, comprising the administration of a non-toxic, vasodilatory amount of a compound of formula (I);
- (h) method for the prophylaxis or treatment of gastric lesions in a mammal, including man comprising the administration of a non-toxic, prophylactic or therapeutic amount of a compound of formula (I);
- (i) (5Z)-5,6-didehydro-9-deoxy-6,9 α -epoxy prostaglandin-F₁₂ and its salts

- (j) method for the promotion of wound healing in a mammal, including man, comprising the administration of a non-toxic wound-treatment amount of a compound of formula (I).

The following examples are provided by way of an illustration of the present invention and should in no way be construed as constituting a limitation thereof.

EXAMPLE 1

Preparation of Prostacyclin

Pig aortas were stripped of adventitia, snap frozen in liquid nitrogen, crushed into a fine powder, resuspended in 0.05 M Tris buffer (pH 7.5) (1:4, w:v) and homogenized at high-speed in a Polytron (KIMMATIC, LUCERNE, SWITZERLAND) homogenizer. The homogenate was centrifuged at 1000 x g for 15 minutes and the resulting supernatant centrifuged again at 10,000 x g for 5 minutes. The 10,000 x g pellet was discarded, while the pellet obtained after centrifugation of the supernatant at 100,000 g for 60 minutes was resuspended in deionized water and lyophilized. An average yield of 150 mg. of aortic microsomal powder (51% protein) per 100 g. of aortic tissue was obtained.

Aortic microsomal powder (5 mg) was incubated with 1 μ g of prostaglandin endoperoxide (PGG₂ or PGH₂) in 0.05M Tris buffer (pH 7.5) (1 ml.) for 2 minutes at 22° C. Enzyme activity was estimated by direct bioassay of the incubation mixture. After incubation of 2 minutes at 22° C. all prostaglandin endoperoxide activity was lost indicated by a lack of contraction of rabbit aorta strips when assayed by the cascade superfusion technique of Vane (Br. J. Pharmac. 23, 369-373, 1964 indicating 100% conversion of PGG₂ or PGH₂).

The Prostacyclin was extracted by the addition of cold dry diethyl ether (1 ml) to the incubation mixture which also stopped the enzymic reaction. The prostacyclin entered the ether phase, which was subsequently separated from the aqueous phase. The ether was evaporated by bubbling nitrogen through it leaving the Prostacyclin which was either dissolved in ice-cold 0.05M Tris buffer (0.5-1 ml.) and immediately used for platelet aggregation studies or was dissolved in anhydrous acetone (1 ml.) and stored at -20° C. for future use.

The anti-aggregatory activity of the extracted Prostacyclin disappeared on boiling (15 seconds) or on standing at 22° C. for 20 minutes. The anti-aggregatory activity of Prostacyclin could be preserved for several days by dissolving the substance in dry acetone and storing at -20° C.

By using rabbit aortas in a similar experiment the same results were obtained.

EXAMPLE 2

Aggregation of platelets in 1 ml. of fresh human platelet rich plasma (PRP) was monitored in a Born aggregometer. An immediate anti-aggregatory effect of the fresh reaction mixture of Example 1 of aortic microsomes with PGH₂ or PGG₂ was observed. The lowest anti-aggregatory concentration was obtained with samples containing 0.5-5 ng. Prostacyclin/ml.

This activity disappeared after leaving the incubation mixture for 20 minutes at 22° C. or by boiling for 15 seconds. Aortic microsomes alone (50 μ g/ml) could induce aggregation in some PRP. The products of spontaneous degradation of PGG₂ (100 ng/ml) had no anti-aggregatory activity.

Diethyl ether extracts of Prostacyclin also inhibited platelet aggregation induced by arachidonic acid and PGG₂. The effective anti-aggregatory concentration of Prostacyclin after storage in ether was from 1 to 10 ng/ml.

EXAMPLE 3

Prostacyclin was injected intravenously and intra-aortically into normotensive anaesthetised rats and the blood pressure and heart rate recorded. Results shows prostacyclin to be a power vasodepressor having 5 times the potency of PGE₂.

EXAMPLE 4

Prostacyclin was found to relax spirally cut strips of coronary artery from the ox. The effect is dose dependent and relaxation can be seen with doses as low as 20 nanograms per 5 ml bath. In isolated hearts from rabbits perfused at constant flow rate by the Langendorf technique, Prostacyclin produced coronary vasodilation.

Prostacyclin relaxed strips of coeliac and mesenteric arteries, but was less potent than PGE₂.

EXAMPLE 5

Prostacyclin was shown to inhibit the formation of gastric lesions induced in rats by indomethacin at doses from 62.5 to 250 µg upon subcutaneous injection.

EXAMPLE 6

A stirred solution of PGF_{2α} methyl ester (50 mg) in ether (1 ml) was treated with sodium bicarbonate (115.0 mg; 10 molecular equivalents) and water (1 ml) and then dropwise during 2 hours with aqueous potassium triiodide (0.7 molar; 0.261 ml). After stirring overnight, the reaction mixture was shaken with ether and aqueous sodium thiosulphate; the ethereal phase was separated, washed with water, dried with magnesium sulphate, and evaporated to leave a yellow gum of 5ξ-iodo-9-deoxy-6ξ,9α-epoxyprostaglandin F_{1α} methyl ester.

A solution of 5ξ-iodo-9-deoxy-6ξ,9α-epoxyprostaglandin F_{1α} methyl ester (100 mg.) in methanolic sodium methoxide prepared from sodium (46 mg.) and dry methanol (0.70 ml.) was set aside under dry nitrogen for 5 hours, then freed from solvent in high vacuum. The residual amorphous solid was washed with benzene, set aside in the air overnight, and stirred with N aqueous sodium hydroxide (0.5 ml.) to give a suspension of colourless fine needles. The crystals were collected, washed with a few drops of N aqueous sodium hydroxide, and dried in the air to give the sodium salt of 9-deoxy-6,9α-epoxy-Δ⁵-prostaglandin F_{1α}. The inhibition of arachidonic acid-induced aggregation of human platelets at a concentration of 0.2 ng./ml. by this salt and its instability in water at acid pH, together with further evidence, is compatible with assignation of the configuration (5Z)-5,6-didehydro-9-deoxy-6,9α-epoxyprostaglandin F_{1α} sodium salt.

The high-resolution ¹³C n.m.r. spectrum of a solution of the crystals in dimethyl sulphoxide-d₆ showed the expected 20 resonances whose chemical shifts were entirely consistent with the chemical structure established for Prostacyclin. No impurity peaks were detected.

EXAMPLE 7

5ξ-Iodo-9-deoxy-6ξ,9α-epoxyprostaglandin F_{1α} methyl ester (500 mg) was stirred with methanolic NaOMe prepared from Na (0.23 g., 10 equivs.) and MeOH (3.5 ml.) under N₂ at room temperature overnight; 1N aq. NaOH (2.5 ml.) was added to the yellow reaction solution to bring about hydrolysis of the ester moiety and, after 2 hours, the methanol was evaporated

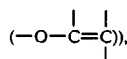
in vacuo at room temperature. The residual aqueous solution gave rise spontaneously to a mass of colourless fine needles of the desired sodium salt (formula (I): R=Na) which was cooled (0°), collected, washed sparingly with 1N aq. NaOH, air-dried, and stored in a stoppered tube; this salt (383mg.) had λ(KBr disc) ν_{max} 1692 cm⁻¹



and twenty ¹³C resonances only were observed (at 182.7 (C-1), 158.2 (C-6), 140.0 and 134.3 (C-13,14), 100.7(C-5), 87.5(C-15), 80.6 and 75.5(C-9,11), 58.0(C-12), 49.0, 45.8, 42.4, 41.9, 37.5, 35.8 (C-18), 31.6, 29.9, 29.3, 26.7(C-19), and 18.4(C-20) ppm from TMS in DMSO-d₆). The product, sodium (5Z)-5,6-didehydro-9-deoxy-6,9α-epoxyprostaglandin F_{1α} (syn. sodium prostacyclin), thus obtained complete inhibited arachidonic acid-induced platelet aggregation (human platelet-rich plasma) at 1 ng./ml. and its profile of biological activity on the rabbit aorta, rabbit coeliac artery, rat stomach strip and rat colon conformed with that of sodium prostacyclin obtained by biosynthesis. After air-drying, the salt has a surface coating of sodium carbonate (ca 3.5% by weight) which protects the vinyl ether moiety against carbondioxide catalysed hydrolysis.

EXAMPLE 8

5ξ-Iodo-9-deoxy-6ξ,9α-epoxyprostaglandin F_{1α} methyl ester was treated with 1,5-diazabicyclo-5-nonene (DBN) at room temperature in the absence of a solvent for a few hours. The DBN and hydrogen iodide were conveniently removed by adsorption on to a column of SiO₂, prepared from a suspension of SiO₂ in EtOAc/Et₃N 50:1, and the vinyl ether was eluted with the same solvent system. I.R. spectroscopy (thin film, ν_{max} 1738 (CO₂Me) and 1696 cm⁻¹



¹H n.m.r. in C₆D₆-Et₃N, 19:1 (84.22, triplet of triplets¹², J_{6,9} and 1.0 Hz (C-5 vinyl proton)), and ¹³C n.m.r. in C₆D₆-Et₃N, 19:1 (distinctive features were resonances at 159.8 (C-1), 155.8 (C-6), 137.2 and 130.6 (C-13,14), 95.3 (C-5), 84.1 (C-15) 77.3 and 72.2 (C-9,11) and 51.1 (Me ester)p.p.m. from TMS).

The vinyl ether (5Z)-5,6-didehydro-9-deoxy-6,9α-epoxyprostaglandin methyl ester, was hydrolysed with aqueous sodium hydroxide to give sodium prostacyclin (formula (I): R=sodium).

What we claim is:

1. A method of treating a mammal for hypertension which comprises parenterly administering an effective anti-hypertensive amount of prostacyclin to said mammal.
2. The method of claim 1 in which the prostacyclin is administered by injection as part of a solution.
3. A method of treating a mammal for hypertension which comprises parenterally administering an effective antihypertensive amount of prostacyclin anion in a liquid to said mammal.
4. The method of claim 3 in which the prostacyclin anion is administered by injection.

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EXHIBIT 7

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DUE DILIGENCE LOG
for IND 16,459 and NDA 20-444

- 08-03-78 Letter to FDA in reference to the BW-FDA meeting scheduled for 1:00 pm on 8/29/78 to discuss plans for our submission of an IND for Prostacyclin (PGI₂), advising that the meeting is primarily intended to share with FDA the current state of our research effort in this area and secondly, to discuss future plans to bring the drug into use in a variety of disease states.
- 05-30-79 Submitted a "Notice of Claimed Investigational Exemption for a New Drug."
- 06-06-79 Letter from FDA acknowledging receipt of our 5/30/79 Notice of Claimed Investigational Exemption for a New Drug.
- 10-20-79 Submitted an amendment to provide for manufacturing and control changes.
- 01-04-80 Telephone call to FDA to report an emergency shipment of prostacyclin to Duke University for an infant with persistent pulmonary hypertension in a newborn.
- 07-02-80 Telephone call to FDA to inform them of the deaths of a newborn infant female, treated on an emergency basis at Duke University Medical Center, July 1 and 2, 1980, for persistent pulmonary hypertension.
- 07-09-80 Telephone call to FDA to discuss the infant with persistent pulmonary hypertension who was treated with prostacyclin at Duke University Medical Center.
- 07-15-80 Amended our IND to provide for the clinical study "Pilot Study of Epoprostenol (Prostacyclin Sodium) in the Treatment of Persistent Pulmonary Hypertension of the Newborn (PPHN) In Neonates Unresponsive to Ventilatory Therapy," to be conducted by George Brumley, M.D.
- 07-23-80 Submitted Progress Report.
- 09-15-80 Amended our IND to provide for the clinical study "Pilot Study of Epoprostenol (Prostacyclin Sodium) In the Treatment of Persistent Pulmonary Hypertension of the Newborn (PPHN) In Neonates Unresponsive to Ventilatory Therapy," to be conducted by Herbert Harned, Jr., M.D.
- 10-20-80 Amended our IND to provide for the clinical study "Pilot Study of Epoprostenol (Prostacyclin Sodium) In the Treatment of Primary Pulmonary Hypertension (PPH) in Adults," to be conducted by Lewis Rubin, M.D., and Bertron Groves, M.D.
- 11-12-80 Submitted an amendment to provide for a revision in the pH of the solution of Prostacyclin for freeze drying from pH 10.5 ± 0.05 to pH 11.4 ± 0.05.
- 11-21-80 Amended our IND to allow for the manufacture of the diluent buffer for the injection at two alternate sites, The Wellcome Foundation, Ltd., Beckenham, U.K., and Glaxo Biologicals Speke, Near Liverpool, U.K.
- 01-28-81 Telephone call to FDA to report an instability problem with prostacyclin; advised FDA that a more detailed letter is forthcoming.

- 04-20-81 Letter to FDA in reference to the 1/28/81 BW-FDA telephone conversation advising FDA of a stability problem with prostacyclin, forwarding a report prepared by the Wellcome Foundation, Ltd., which fully describes the background of the stability problem.
- 04-20-81 Meeting with FDA to discuss (1) formal notification to FDA of the problem of loss of potency in one lot of drug, our investigation, and our conclusion (2) discussion of a preliminary review of our IND by Dr. Weiss.
- 05-28-81 Telephone conversation with FDA regarding their interest in prostacyclin as a diagnostic tool in determining the reversibility of pulmonary hypertension.
- 06-02-81 In reference to our letter of 4/20/81 concerning a stability problem with prostacyclin, submitted original graphs of the results of the Pyrogallol experiment to show the oxygen levels.
- 08-31-81 Submitted Progress Report.
- 11-02-81 Telephone conversation with the FDA to request permission to administer prostacyclin to a patient with primary pulmonary hypertension, entered in Dr. Bertram Groves clinical study.
- 11-05-81 Letter to FDA regarding recent observations by the Upjohn Company of alterations in bone growth in dogs and neonates following administration of PGE₁, PGE₂, and PGF₂.
- 01-12-82 Telephone call to Dr. Eugenio Triantafyllidis (FDA) to request permission to treat patient with pulmonary hypertension.
- 01-18-82 Telephone call to FDA to request permission to treat a patient with primary pulmonary hypertension.
- 02-15-82 Submitted an amendment to our IND to provide for the use of a 0.22 μ m sterile membrane filter.
- 06-24-82 Amended our IND to provide for a clinical study entitled "evaluation of Epoprostenol Sodium and Subsequent Therapy in the Treatment of Primary Pulmonary Hypertension (PPH) in Adults," to be conducted by Bertron Groves, M.D.
- 07-27-82 Amended our IND to provide for the clinical study entitled "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Pulmonary Vasoreactivity in Patients with Primary Pulmonary Hypertension (PPH).
- 07-27-82 In addition, we wish to amend our IND to provide for the clinical study entitled "Evaluation of Epoprostenol sodium and Subsequent Therapy in the Treatment of Primary Pulmonary Hypertension (PPH) in Adults," to be conducted by Lewis J. Rubin, M.D.
- 08-10-82 Amended our IND to provide for the emergency use of Prostacyclin Sodium Salt by Bertron Groves, M.D., of University of Colorado School of Medicine, Denver, Colorado and Hugo D. Mortenogro, M.D., Cleveland VA Medical Center, Cleveland, Ohio, in the treatment of patients with primary pulmonary hypertension.
- 08-11-82 Submitted Progress Report

- 09-02-82 Amended our IND to register Robert Mellins, M.D., as an investigator for the clinical study entitled "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Pulmonary Vasoreactivity in Patients with Primary Pulmonary Hypertension (PPH)," submitted to FDA 7-27-82.
- 09-07-82 Telephone conversation with Doralie Segal of FDA regarding Dr. Herbert B. Hectman's clinical study.
- 10-01-82 Amended our IND to provide for an amendment to the clinical protocol entitled "Evaluation of Epoprostenol Sodium and Subsequent Therapy in the Treatment of Primary Pulmonary Hypertension (PPH) in Adults," being conducted by Lewis J. Rubin, M.D., and Bertron Groves, M.D., submitted 8-27-82 and 6-24-82, respectively.
- 04-07-83 Amended our IND to register Syed Mohiuddin, M.D., as an investigator for the clinical study (21-02) entitled "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Pulmonary Vasoreactivity in Patients with Primary Pulmonary Hypertension (PPH)," submitted to FDA 7-27-82.
- 05-17-83 Amended our IND to provide for revisions to the clinical study 21 entitled "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Pulmonary Vasoreactivity in Patients with Primary Pulmonary Hypertension," being conducted by Robert Mellins, M.D. and Syed Mohiuddin, M.D.
- 10-17-83 Amended IND to register Bertron Groves, M.D. as investigator for the study entitled "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Pulmonary Vasoreactivity in Patients with Primary Pulmonary Hypertension." Study No. 21-05.
- 12-05-83 Amended IND to provide for the following registration of Lewis J. Rubin, M.D. as an investigator for the clinical study entitled "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Pulmonary Vasoreactivity in Patients with Primary Pulmonary Hypertension (PPH)," submitted to FDA on July 27, 1982, and amended on May 17, 1983.
- 04-25-84 Amended IND to provide for registration of Kenneth Moser, M.D. as an investigator for clinical study 21, "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Pulmonary Vasoreactivity in Patients with Primary Pulmonary Hypertension (PPH)," submitted 7/27/82.
- 01-27-84 Submitted an amendment to our IND to provide for an updating of the synthetic route for the new drug substance.
- 03-15-84 Submitted a copy of the minutes of the End-of-Phase II Meeting held with FDA 11/21/83, and a copy of Technical Report on the 30-day dog study, entitled "Formation and Reversibility of Hematological Changes in Beagle Dogs," conducted by the Upjohn Company.

- 07-09-84 Submitted Progress Report.
- 07-10-84 Amended IND to provide for clinical study 29, "Evaluation of Epoprostenol Sodium Effects on Pulmonary Vascular Resistance, Pulmonary Function and Exercise Tolerance in Adult Patients With Cor Pulmonale from Chronic Obstructive Pulmonary Disease," to be conducted by Lewis J. Rubin, M.D. In addition, we wish to amend IND to provide for a change in principal investigator from Richard Foley, M.D. to Catherine Thompson, M.D. for the following clinical studies:
- 11-07-84 Amended IND to provide for:
Amendment of clinical study 29, "Evaluation of Epoprostenol Sodium on Pulmonary Vascular Resistance, Pulmonary Function, and Exercise Tolerance in Adult Patients with Cor Pulmonale from Chronic Obstructive Pulmonary Disease," being conducted by Lewis Rubin, M.D., to allow additional platelet aggregation tests. The protocol for this study and a completed Form FD 1572/1573 for Dr. Rubin were submitted to FDA on July 10, 1984.
- 02-07-85 Amended IND to provide for revisions to clinical study 29, being conducted by Lewis Rubin, M.D. (Protocol submitted to FDA 7-10-84 and amended 11-7-84).
- 05-15-85 Conversations with FDA regarding primary pulmonary
and hypertension (PPH) indication for prostacyclin requesting
05-24-85 additional data.
- 08-14-85 Submitted Progress Report.
- 08-15-85 Amended IND to register Robyn Barst, M.D. as an investigator for clinical study 21, "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Pulmonary Vasoreactivity in Patients with Primary Pulmonary Hypertension (PPH)," submitted to FDA on July 27, 1982, and amended on May 17, 1983.
- 12-24-85 Amended our IND to register Jeffrey Dunn as an investigator for clinical study 21, submitted to FDA on July 27, 1982, and amended on May 17, 1983.
- 02-27-86 Amended our IND to register Lewis J. Rubin, M.D., as investigator for clinical study 29, sites 03 and 04, submitted to FDA on 7/10/84, and amended on 11/7/84 and 2/7/85.
- 04-28-86 Amended our IND to provide for a change in principal investigator for study 21, submitted to FDA on 07/27/82 and amended on 5/17/83; Neal H. Cohen, M.D., a co-investigator will assume responsibility for this study.
- 10-06-86 Submitted a progress report.

- 10-31-86 Amended IND to provide for revisions to clinical study 20, "Evaluation of Epoprostenol Sodium and Subsequent Therapy in the Treatment of Primary Pulmonary Hypertension (PPH) in Adults," being conducted by Lewis Rubin, M.D. and Bertron Groves, M.D. The protocol for this study was submitted on June 24, 1982.
- 04-23-87. Amended IND to provide for clinical study 36, "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," to be conducted by Robyn J. Barst, M.D
- 04-28-87 Telephone call to FDA informing them of the death of patient #101 who was enrolled in study 36, "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Robyn Barst, M.D.
- 05-04-87 Submitted an adverse experience report on a patient (#101, PLC) who expired following treatment under clinical study 36, "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Robyn Barst, M.D. Also submitted information on a patient who expired following treatment with FLOLAN during an attempted cardiopulmonary resuscitation.
- 05-07-87 Telephone call to FDA to clarify procedure for submission of adverse experience reports during NDA review; ADR's should be submitted to both the IND and NDA until NDA is approved.
- 06-18-87 Amended IND to provide for revisions to clinical study 36, "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients." In addition, registered Lewis Rubin, M.D. as an investigator for study 36. The protocol for this study was submitted April 23, 1987.
- 07-01-87 Amended IND to provide for clinical study 35, "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes I and II Primary Pulmonary Hypertension Patients," to be conducted by Lewis Rubin, M.D.
- 07-31-87 Submitted adverse experience report on a patient (#02, CJD), who experienced embolic cerebrovascular accident while being treated under protocol 36, "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Lewis Rubin, M.D.
- 08-21-87 Telephone call from FDA regarding adverse experience (cerebrovascular accident) submitted on July 31, 1987. We were requested to notify investigators of this experience.
- 08-26-87 Amended IND to provide for clinical study 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," to be conducted by Lewis Rubin, M.D.

- 09-02-87 Letter from FDA confirming telephone conversation of August 21, 1987 and requesting that we notify investigators of adverse experience (cerebrovascular accident) submitted July 31, 1987.
- 09-04-87 Submitted a follow-up to our May 4, 1987 submission concerning the death of patient #101 (PLC) who was being treated under protocol 36, "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III or IV Primary Pulmonary Hypertension."
- 09-11-87 Telephone call from FDA with concerns regarding protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," submitted on August 26, 1987.
- 09-15-87 Telephone call to FDA to review the status of patient entry and outcome for studies 35, 36 and 37.
- 09-25-87 Letter from FDA requesting that we submit additional safety report summaries at three month intervals for study 37-01, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension."
- 09-28-87 Submitted copy of our letter to investigators notifying them of adverse experience concerning patient #02, in response to FDA letter of September 2, 1987.
- 10-22-87 Amended IND to register Alfred Fishman, M.D. and Harold
001 Palewsky, M.D. as investigators for the following clinical studies:

Protocol 35 - "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes I and II Primary Pulmonary Hypertension Patients," submitted on July 1, 1987.

Protocol 36 - "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," submitted on April 23, 1987 and amended on June 18, 1987.
- 10-30-87 Submitted a copy of letter to FDA that had been forwarded to all
002 investigators concerning safety precautions, re: protocols 35, 36, and 37, as a follow-up to our September 28, 1987 submission.
- 11-09-87 Submitted three-month update covering period up to October 1,
003 1987 for the following clinical studies as requested by FDA in letter of September 25, 1987:

Protocol 35 - "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes I and II Primary Pulmonary Hypertension Patients"

Protocol 36 - "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients"

Protocol 37 - "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension"

- 11-16-87
004 Amended IND to provide for clinical study 40, "Evaluation of the Hemodynamic Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," to be conducted by Matthew Horn, M.D., and Kenneth Moser, M.D.
- 12-21-87
005 Amended IND to register Lewis Rubin, M.D. as an investigator for clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure." Protocol for study was submitted on November 16, 1987 (004).
- 01-21-88
006 Amended IND to register Mitchell Friedman, M.D. as an investigator for clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," submitted on November 16, 1987. Also registered Robyn Barst, M.D. as an investigator for clinical study 21, "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Pulmonary Vasoreactivity in Patients with Primary Pulmonary Hypertension," submitted on July 27, 1982 and amended on May 17, 1983. Dr. Barst is replacing Thomas Starc, M.D.
- 01-29-88
007 Submitted the second three-month safety update for studies 35, 36 and 37 covering the period from October 1, 1987 to December 31, 1987.
- 02-22-88
008 Amended IND to register Michael D. McGoon, M.D. as an investigator for clinical study 35, "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes I and II Primary Pulmonary Hypertension Patients," submitted on July 1, 1987 and clinical study 36, "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," submitted on April 23, 1987 and amended on June 18, 1987. In addition, registered Alfred Fishman, M.D. and Harold Palevsky, M.D. as investigators for clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," submitted on November 16, 1987.
- 03-08-88
009 Submitted annual report.
- 03-25-88
010 Amended IND to provide for revisions to clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," submitted on November 16, 1987 (Serial No. 004). The protocol is being conducted by the following investigators:
- | | |
|-------------------------|----------------------|
| Lewis Rubin, M.D. | Alfred Fishman, M.D. |
| Harold Palevsky, M.D. | Kenneth Moser, M.D. |
| Mitchell Friedman, M.D. | Edgar Caldwell, M.D. |
| William Williams, M.D. | |

Also registered Michael McGoon, M.D. as an investigator for clinical study 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in patients with Primary Pulmonary Hypertension," submitted on August 26, 1987.

03-31-88 Telephone call to FDA to report the death of patient #17 who expired while being treated under clinical study 36, "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Robyn Barst, M.D.

04-21-88 Submitted an adverse experience report on a patient (#18, JAF)
011 who experienced flushing, faintness, nausea, hypotension and bradycardia while being treated under protocol 36, "Multicenter Evaluation of Long-Term FLOLAN in New York Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Lewis Rubin, M.D.

04-26-88 Submitted the third three-month safety update for the
012 following clinical studies:

Protocol 35 - "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes I and II Primary Pulmonary Hypertension Patients"

Protocol 36 - "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients"

Protocol 37 - "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension"

04-27-88 Submitted summary information on patient #17 (VS) who
013 expired while being treated under clinical study 36, "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Robyn Barst, M.D.

05-05-88 Submitted an adverse experience report on patient #01 (REC)
014 who experienced cough syncope and bronchitis while being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D.

05-23-88 Amended IND to register Frederick Glauser, M.D., Paul Fairman,
016 M.D. and Curt Sessler, M.D. as investigators for clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," submitted on November 16, 1987 (Serial No. 004).

06-27-88 Amended IND to register Keith Mansel, M.D. and James E.
017 Griffith, M.D. as investigators for clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," submitted on November 16, 1987 (Serial No. 004).

- 07-08-88
018 Submitted an adverse experience report on patient #18 (JAF) who experienced syncope while being treated under protocol 36, "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Lewis Rubin, M.D.
- 07-08-88 Telephone call to FDA informing them of patient #01 (REC) who experienced skin and eye photosensitivity while being treated under clinical study 37-01, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D.
- 07-11-88 Telephone call to FDA informing them of patient #16 (DMC) who experienced an increase in pulmonary arterial pressure while being treated under protocol 36, "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Michael McGoon, M.D.
- 07-15-88
019 Submitted an adverse experience report on patient #01 (REC) who experienced skin and eye photosensitivity while being treated under protocol 37-01, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D.
- 07-20-88
020 Submitted an adverse experience report on patient #16 (DMC) who experienced an increase in pulmonary arterial pressure (PAP) while enrolled in clinical study 36, "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Michael McGoon, M.D.
- 07-27-88
021 As a follow-up to our telephone call of March 31, 1988, and letter of April 17, 1988, submitted an autopsy report on patient #17 (VS) who expired while being treated under protocol 36, "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Robyn Barst, M.D.
- 07-28-88
022 Amended IND to register Ronald Pearl, M.D., Ph.D., Steve Jenkinson, M.D., Charles Bryan, M.D., Warren Summer, M.D. and Bennett deBoisblanc, M.D. as investigators for clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," submitted on November 16, 1987 (Serial No. 004).
- 08-10-88
023 Submitted annual report.

- 09-08-88
026 Submitted an adverse experience report on patient #06 (DMC) being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Michael McGoon, M.D.
- 09-13-88 Telephone call to FDA to explain our delay in supplying the last 90-day safety report for primary pulmonary hypertension.
- 09-14-88
028 Submitted the fourth three-month safety update for the following studies:

Protocol 35 – Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes I and II Primary Pulmonary Hypertension Patients

Protocol 36 – Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients

Protocol 37 – Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension.
- 09-22-88
029 Amended IND to register Kenneth M. Moser, M.D. and Kent Kapitan, M.D. as investigators for clinical study 36, "Multicenter Evaluation on Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Patients," submitted on April 23, 1987 and amended on June 18, 1987. Also, registered James Williams, M.D. and Kenneth Weir, M.D. as investigators for clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," submitted November 16, 1987 (Serial No. 004).
- 10-20-88 Telephone call to FDA to report an adverse event in which patient #12 experienced pulmonary edema while being treated under protocol 36.
- 10-24-88
030 Amended IND to register Michael Nochomovitz, M.D. as an investigator for clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease patients with Acute Respiratory Failure," submitted on November 16, 1987 (Serial No. 004).
- 10-24-88 Telephone call to FDA to report the death of patient #04 being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D.
- 10-25-88
031 As a follow-up to the submission of September 8, 1988, submitted the hospital record for patient #06 (DMC) being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Michael McGoon, M.D.

- 10-25-88 Amended IND to provide for revisions to clinical study 37,
032 "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D. and Michael McGoon, M.D. The protocol for this study was submitted on August 26, 1987.
- 11-03-88 Telephone call to FDA to report that patient #06 (DMC)
experienced cramps, light-headedness, rapid pulse, lowered blood pressure and subsequent non-responsiveness while being treated under protocol 37, being conducted by Michael McGoon, M.D.
- 11-08-88 Submitted an adverse experience report on patient #12 (MBS)
033 who experienced severe, life-threatening pulmonary edema while enrolled under protocol 36, "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Michael McGoon, M.D.
- 11-11-88 Submitted an adverse experience report on patient #01 (WRS)
034 who expired while being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D.
- 11-15-88 Submitted an adverse experience report on patient #06 (DMC)
035 who experienced altered mental status and hypotension while being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Michael McGoon, M.D.
- 12-08-88 Amended IND to provide for revisions to clinical study 40,
036 "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," submitted on November 16, 1987 (Serial No. 004).
- 12-30-88 Amended IND to register Harold I. Palevsky, M.D. as an
037 investigator for clinical study 21, "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Primary Vasoreactivity in Patients with Primary Pulmonary Hypertension and Selected Patients with Secondary Pulmonary Hypertension," submitted on July 27, 1982 and amended on May 19, 1983.
- 01-10-89 Submitted the fifth three-month safety update for the following
038 studies:

Protocol 35 - "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes I and II Primary Pulmonary Hypertension Patients."

Protocol 36 - "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients."

Protocol 37 – "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusion in Patients with Primary Pulmonary Hypertension."

- 01-20-89
039 Amended IND to register Frank Lewis, M.D. as an investigator for clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," submitted on November 16, 1987 (Serial No. 004) and amended on December 8, 1988 (Serial No. 036).
- 03-13-89 Telephone call to FDA to report an adverse event experience by patient #6 who became cyanotic, collapsed and developed a seizure while being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusion in Patients with Primary Pulmonary Hypertension," being conducted by Michael McGoon, M.D.
- 03-17-89
040 In accord with FDA letter of September 25, 1987, submitted the sixth three-month safety update which covers the period from October 1, 1988 to December 31, 1988 for the following studies:

Protocol 36 – "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients"

Protocol 37 – "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusion in Patients with Primary Pulmonary Hypertension"
- 03-29-89
041 Amended IND to register Stephen Jenkinson, M.D. and Charles Bryan, M.D. as investigators for clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," submitted on November 16, 1987 (Serial No. 004) and amended on December 8, 1988 (Serial No. 036).
- 03-30-89
042 Submitted an adverse experience report on patient #6 (DMC) who experienced cyanosis, absence of respirations, seizure activity and loss of consciousness while being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusion in Patients with Primary Pulmonary Hypertension," being conducted by Michael McGoon, M.D.
- 04-26-89 Telephone call to FDA to report that patient #05, enrolled in study 37, had an AutoSyringe pump malfunction, and developed SOB and severe dyspnea.
- 05-11-89
043 Submitted an adverse experience report on patient #05 (JAF) who experienced a sudden onset of dyspnea while being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D.
- 05-31-89
044 Submitted the seventh three-month safety update for the following studies:

Protocol 36 – "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients"

Protocol 37 – "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusion in Patients with Primary Pulmonary Hypertension"

- 07-11-89 Telephone conversation with FDA in which we were informed that the 3-month safety updates for FLOLAN in treating patients with primary pulmonary hypertension could be discontinued.
- 07-26-89 Letter to FDA confirming telephone agreement that 3-month
045 safety updates for protocols 35, 36 and 37 would be discontinued.
- 08-10-89 Submitted annual report.
046
- 09-08-89 Submitted an IND Safety Report on patient #24 (DPM) who
047 expired while being treated under protocol 21, 11 Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Primary Vasoreactivity in Patients with Primary Pulmonary Hypertension and Selected Patients with Secondary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D.
- 09-25-89 Letter to FDA notifying them of additional patient recruitment (3) in Protocol 36.
- 11-07-89 Telephone call to FDA to inform them that patient #28 expired while being treated under protocol 36 being conducted by Robyn Barst, M.D.
- 11-28-89 Telephone call to FDA to inform them of the death of patient #9 who was being treated under protocol 36 being conducted by Lewis Rubin, M.D.
- 12-13-89 Submitted IND Safety Report on patient #28 (AB) who expired
049 while being treated under protocol 36, "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Robyn Barst, M.D.
- 01-22-90 Submitted IND Safety Report on patient #09 (JAW) who expired
050 while enrolled in the control group of clinical study 36, "Multicenter Evaluation of Long-Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Lewis Rubin, M.D.
- 02-19-90 Amended IND to register Robyn J. Barst, M.D. as an investigator
052 for clinical study 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," submitted on August 26, 1987 and amended on October 25, 1988 (032).
- 02-20-90 Telephone call to FDA to report the death of patient #2 (PPH) who expired while being treated under protocol 37.
- 03-06-90 Telephone call to FDA to inform them of the publication of an article describing FLOLAN use in Primary Pulmonary Hypertension which is scheduled to appear in the April issue of Annals of Internal Medicine and to discuss the results of a television interview on CNN with one of our investigators and one of the patients receiving FLOLAN for PPH.

- 03-14-90 Submitted data to FDA in preparation for a meeting tentatively scheduled for April 4, 1990 to discuss recent results describing the use of FLOLAN in treating patients with Primary Pulmonary Hypertension and the implications of these results on the possible submission of a Treatment IND.
- 03-29-90 In reference to our submission of March 14, 1990 which provided
054 background material for the meeting of April 4, 1990, submitted a tentative list of Burroughs Wellcome attendees for that meeting and a reprint of an article from the 1987 British Heart Journal detailing FLOLAN's use in Primary Pulmonary Hypertension.
- 03-29-90 Submitted a written report and a report on the stoppage of the
055 pump concerning patient #2 (CJD) who expired while being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusion in Patients with Primary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D.
- 03-30-90 Telephone call to FDA to inform them of the death of patient #36
(RMD) being treated under protocol 21 by Dr. Lewis Rubin.
- 04-05-90 Letter to FDA authorizing them to refer to our IND 16,459 and to
056 our pending NDA 19-607 on behalf of the Upjohn Company, Kalamazoo, Michigan.
- 04-19-90 Telephone call from FDA to discuss FLOLAN's development as a
diagnostic agent in primary pulmonary hypertension.
- 04-25-90 Submitted an IND Safety Report on patient #36 (RMD) who
057 experienced cardiopulmonary arrest and subsequently expired while being treated under protocol 21, "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Pulmonary and Vasoreactivity in Patients with Primary Pulmonary Hypertension (PPH)" being conducted by Lewis Rubin, M.D.
- 05-09-90 Letter from FDA with their summary of the April 4, 1990,
meeting.
- 05-23-90 Submitted a report on patient #01 (REC) who experienced
058 catheter sepsis, bacteremia, and microscopic hematuria while being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusion in Patients with Primary Pulmonary Hy[ertension]," being conducted by Lewis Rubin, M.D.
- 05-23-90 Submitted a report on patient #15 (RV) who experienced
059 infection at catheter site while being treated under protocol 37, ""Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusion in Patients with Primary Pulmonary Hy[ertension]," being conducted by Robyn Barst, M.D.
- 06-12-90 Telephone call to FDA to inform them of the death of patient #08
(HLC) who suffered a cardiac arrest and subsequently expired while being treated under protocol 37.

- 06-28-90 060 In reference to meeting held on April 4, 1990, to discuss the use of FLOLAN in treating patients with Primary Pulmonary Hypertension, submitted our minutes of that meeting and comments regarding the FDA minutes of that meeting, together with a copy of the videotape of the CNN broadcast concerning FLOLAN.
- 06-28-90 Submitted our minutes of the April 4, 1990, meeting and comments regarding the FDA minutes of that meeting.
- 07-02-90 061 Submitted an IND Safety Report on patient #08 (HLC) who suffered acardiac arrest and subsequently expired while being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusion in Patients with Primary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D.
- 07-10-90 In reference to our Letter to the FDA of June 28, 1990 regarding the material on the videotape of the CNN broadcast, letter from FDA requesting a detailed, up-to-date report on the patient presented on that broadcast.
- 08-02-90 062 Submitted the case history for the patient presented on the videotape of the CNN broadcast as requested in the FDA letter of July 10, 1990.
- 09-05-90 Memo to FDA detailing three separate datasets utilized in these analyses: NIH NHLBI PPH National Registry Data; Papworth Hospital (UK) Data; and BW FLOLAN Protocols 35, 36, 37 Data.
- 09-18-90 063 Submitted data which provides evidence that FLOLAN is safe and effective therapy that prolongs survival and improves exercise capacity in NYHA Class III and IV patients with PPH; requested meeting to discuss possible Treatment IND.
- 10-05-90 064 In reference to telephone conversation of October 4, 1990, submitted summary information on Patient #16, (JJD) who experienced symptoms probably due to infusion pump failure while being treated under protocol 37, "O(pen Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D.
- 10-26-90 Telephone call to FDA regarding the review status of our September 18, 1990 submission that was in support of a possible treatment IND and to discuss the Subpart E status for this drug.
- 10-26-90 thru 01-10-91 Telephone contacts with FDA regarding the status of their review of our September 18, 1990 submission in support of the Treatment IND.
- 10-31-90 Telephone call from FDA to inform us that Subpart E status for FLOLAN would be granted and their review of our submission regarding the filing of a Treatment IND had not been completed.
- 11-05-90 In response to our request of June 28, 1990, letter from FDA informing us that FLOLAN qualifies for the special procedures designed to expedite the development, evaluation, and marketing of new therapies as delineated in Subpart E.

- 11-09-90 Amended IND to provide for revisions in the manufacturing and
065 controls data for the use in clinical trials with FLOLAN Sterile Powder.
- 11-13-90 Letter from FDA requesting a progress report.
- 12-20-90 Submitted IND Safety Reports on the following patients being
067 treated under protocol 37 by Lewis Rubin, M.D.:
Patient #16 (JJD) who experienced shortness of breath, blackout, tightness in chest and diarrhea (9/3/90); and weakness, clamminess, faintness, shortness of breath, diarrhea, pallor, inability to move and glazed eyes (9/26/90); and patient #1 (REC) who experienced hypotension and grand mal seizure (following inadvertent overdose of FLOLAN).
- 12-21-90 Submitted IND Safety Reports on the following patients being
068 treated under protocol 37 by Lewis Rubin, M.D.:
Patient #07 (ECT) who experienced the loss of short term memory; and patient #13 (LLW) who experienced a symptom complex which included the inability to move, lightheadedness, anxiety, left arm pain, diarrhea, pallor, clamminess, diaphoresis and tachycardia.
- 01-14-91 Conference call with FDA to discuss their proposal that we file a
Treatment IND and follow the mortality in additional patients under the Treatment IND.
- 01-17-91 Submitted an Adverse Experience Report on Patient #07 (ECT)
070 who experienced shortness of breath/dyspnea, diarrhea, blackout/disorientation, vomiting and lightheadedness while being treated under protocol 37 (conducted by Lewis Rubin.)
- 01-18-91 Submitted Adverse Experience Reports on the following patients
071 being treated under protocol 37:
Patient #17 (KP) who experienced sepsis while being treated by Robyn Barst, M.D.; and patient #01 (REC) who experienced a serious thrombosis near or in the indwelling catheter and sepsis while being treated by Lewis Rubin, M.D.
- 01-22-91 Submitted Annual Report which covers the period of April 1,
072 1989 through March 31, 1990.
- 01-22-91 As a follow-up to conference call with FDA on January 14, 1991,
telephone call to FDA to obtain proposed dates they could meet with us to discuss further the Treatment IND.
- 01-23-91 In response to our January 22, 1991 call regarding possible
meeting dates to discuss the treatment IND, telephone call from FDA requesting that we submit a written request for a meeting, a list of the BW Co. attendees and any new information we intend to present to them.
- 01-28-91 Telephone call to FDA informing them that we would be
submitting a written request for a meeting and a list of meeting attendees as requested by the FDA on January 23, 1991.
- 01-30-91 Telephone call from FDA informing us that the meeting date to
discuss the Treatment IND was scheduled on February 14, 1991.

- 01-31-91 Telephone call to FDA to confirm that February 14, 1991 would be a suitable date for the meeting.
- 02-06-91 Letter to FDA confirming the meeting scheduled for February 14, 1991 and submitting 1) a list of attendees; 2) a proposed agenda for the meeting; and 3) Summary of Updated Survival Data in preparation for the meeting.
- 02-06-91 Letter to FDA confirming the meeting scheduled for February 14, 1991.
- 074
- 02-13-91 Letter from FDA requesting a progress report.
- 02-18-91 Submitted an Adverse Experience Report on patient #17 (KP) who experienced sepsis, hypotension, low cardiac output and nausea while enrolled under protocol #17, being conducted by Robyn Barst, M.D.
- 073
- 04-03-91 Submitted a copy of our minutes of FDA meeting held on February 14, 1991, regarding the use of FLOLAN in the treatment of PPH.
- 079
- 04-04-91 Telephone conversation with FDA to clarify our position on a request received by the FDA from a Dr. Prince for emergency treatment of a patient (KW) with PPH.
- 07-12-91 Amended IND to provide for an additional 1.5 mg strength of FLOLAN Sterile Powder.
- 090
- 07-31-91 As a follow-up to meeting of February 14, 1991, letter to FDA requesting a meeting to discuss our future development plans for FLOLAN in the treatment of primary pulmonary hypertension patients; provided summary data in preparation for the meeting.
- 08-16-91 Letter from FDA enclosing their summary of the meeting held on February 14, 1991.
- 19 Aug 91 Minutes of meeting with FDA to discuss 1) toxicology requirements for chronic use of FLOLAN in patients with PPH and CHF; 2) the proposed protocol for a second exercise tolerance study to support NDA approval for PPH, and 3) labeling specifications for the intravenous delivery system.
- 09-06-91 In reference to FDA letter of August 16, 1991, summarizing the February 14, 1991 meeting, letter to FDA to requesting clarification of the agency's position on the inclusion of mortality claims in the label if the drug is approved for PPH on the basis of a second exercise study.
- 097
- 09-11-91 In reference to meeting with FDA on August 19, 1991 submitted a revised protocol 46, "Multicenter, Open-Label, Randomized, Parallel Comparison of the Safety and Efficacy of Chronic FLOLAN Infusions Plus Conventional Therapy Alone in Patient with Severe Primary Pulmonary Hypertension," to be conducted by Robyn Barst, M.D. The protocol reflects the following changes: 1) randomization assignment will be stratified by center, NYHA Class and vasodilator use at baseline; and 2) conventional therapy medications will be held constant unless clinical necessity dictates otherwise.
- 099

- 10-04-91 Amended IND to provide for revisions to clinical study 37,
101 "Open Multicenter Evaluation of the Effects of Chronic FLOLAN
Infusions in Patients with Primary Pulmonary Hypertension".
The protocol is being amended to allow use of a larger vial of
FLOLAN Powder, use of an in-line filter, storage of vials at room
temperature and preparation of higher concentrations of
FLOLAN solutions.
- 10-29-91 Amended IND to register Robert Bourge, M.D. and Lewis Rubin,
103 M.D. as an investigator to conduct clinical study 46, "A
Multicenter, Open-Label, Randomized, Parallel Comparison of
the Safety and Efficacy of Chronic FLOLAN Infusions Plus
Conventional Therapy to Conventional therapy Alone in Patients
with Severe Primary Pulmonary Hypertension: A Twelve-Week
Study".
- 11-07-91 In response to our letter of September 6, 1991 requesting
clarification of the agency's position on the inclusion of mortality
claims in the label, letter from FDA stating that they would not
rule out some mention of mortality results in the labeling if the
drug is approved, but, mortality would not be promotable as an
efficacy claim.
- 11-11-91 In reference to telephone conversation of September 23, 1991, as
105 requested, submitted additional details on the randomization
procedure to be used in the FLOLAN exercise trial in patients
with PPH.
- 11-12-91 Submitted Annual Report which covers the period of April 1,
104 1990 thru March 31, 1991.
- 20 Nov 91 Submitted to FDA an Information Package in preparation for the
End of Phase II meeting scheduled for 12 Dec 91.
- 12-05-91 Amended IND to provide for clinical study 47, "A Multicenter,
106 Open Evaluation of the Safety of Chronic FLOLAN Infusions
Plus Conventional Therapy in Patients with Severe Primary
Pulmonary Hypertension: A Continuation Study," to be
conducted by Robyn Barst, M.D.
- 12-09-91 Letter to FDA to confirm the meeting scheduled for December 12
and submitted copies of our proposed agenda, a list of persons
attending and a summary of the changes made in the draft
clinical protocol previously provided to FDA.
- 12-13-91 Amended IND to register Neil Ettinger, M.D., Victor
107 Tapson, M.D., Anthony Killian, M.D., Ph.D. and Stuart Rich,
M.D. as investigators to conduct clinical study 46, "A
Multicenter, Open-Label, Randomized, Parallel Comparison of
the Safety and Efficacy of Chronic FLOLAN Infusions Plus
Conventional Therapy to Conventional Therapy Alone in
Patients with Severe Primary Pulmonary Hypertension: A
Twelve-Week Study."

- 12-31-91
108 Amended IND to register David Badesch, M.D., FACP, FCCP, Bertron Groves, M.D. and Edgar Caldwell, M.D. to conduct clinical study 46, "A Multicenter, Open-Label, Randomized, Parallel Comparison of the Safety and Efficacy of Chronic FLOLAN Infusions Plus Conventional Therapy to Conventional Therapy Alone in Patients with Severe Primary Pulmonary Hypertension: A Twelve-Week Study" and Lewis Rubin, M.D., to conduct clinical study 47, "A Multicenter, Open Evaluation of the Safety of Chronic FLOLAN Infusions Plus Conventional Therapy in Patients with Severe Primary Pulmonary Hypertension: A Continuation Study."
- 01-27-92
109 Amended IND to register William Clarke, M.D. as an investigator for clinical study 46, "A Multicenter, Open-Label, Randomized, Parallel Comparison of the Safety and Efficacy of Chronic FLOLAN Infusions Plus Conventional Therapy to Conventional Therapy Alone in Patients with Severe Primary Pulmonary Hypertension: A Twelve-Week Study" and Robert Bourge, M.D. for clinical study 47, "A Multicenter, Open Evaluation of the Safety of Chronic FLOLAN Infusions Plus Conventional therapy in Patients with Severe Primary Pulmonary Hypertension: A Continuation Study".
- 02-27-92
110 Amended IND to register the following investigators to conduct clinical study 46, "Multicenter, Open-Label, Randomized, Parallel Comparison of the Safety and Efficacy of Chronic FLOLAN Infusions Plus Conventional Therapy to Conventional Therapy alone in Patients with Severe Primary Pulmonary Hypertension: A Twelve-Week Study"
- Bruce Brundage, M.D. Barry Uretsky, M.D.
Michael McGoon, M.D. Warren Summer, M.D.
Srinivas Murali, M.D., FACC, FACP
- 5 Mar 92 Telephone call to FDA regarding the death of patient #15999 (MVP) who experienced a pneumothorax secondary to an attempted Schwann-Ganz catheterization and subsequently expired while being treated under protocol 46 by Dr. Michael McGoon.
- 03-17-92
111 Amended IND to register David Langleben, M.D., Montreal, Canada as an investigator for clinical study 46, "A Multicenter, Open-Label, Randomized, Parallel Comparison of the Safety and Efficacy of Chronic FLOLAN Infusions Plus Conventional Therapy to Conventional Therapy alone in Patients with Severe Primary Pulmonary Hypertension: A Twelve-Week Study".
- 03-31-92
112 In reference to telephone conversation of March 5, 1992, submitted IND Safety Report on patient #15999 (MVP) who experienced a pneumothorax secondary to an attempted Schwann-Ganz catheterization and subsequently expired while being treated under protocol 46.

- 04-01-92 Amended IND to register Spencer Koerner, M.D., and Nicholas
113 Hill, M.D. as investigators to conduct clinical study 46, "A Multicenter, Open-Label, Randomized, Parallel Comparison of the Safety and Efficacy of Chronic FLOLAN Infusions Plus Conventional Therapy to Conventional Therapy alone in Patients with Severe Primary Pulmonary Hypertension: A Twelve-Week Study" and Victor Tapson, M.D., David Badesch, M.D., Bertron Groves, M.D. and Stuart Rich, M.D. as investigators to conduct clinical study 47, "A Multicenter, Open-Evaluation of the Safety of Chronic FLOLAN Infusions Plus Conventional Therapy in Patients with Severe Primary Pulmonary Hypertension: A continuation Study".
- 04-21-92 Amended IND to register Cesar Keller, M.D. as a investigator to
114 conduct clinical study 46, "A Multicenter, Open-Label, Randomized, Parallel Comparison of the Safety and Efficacy of Chronic FLOLAN Infusions Plus Conventional Therapy to Conventional Therapy alone in Patients with Severe Primary Pulmonary Hypertension: A Twelve-Week Study" and William Clarke, M.D. as a investigator to conduct clinical study 47, "A Multicenter, Open-Evaluation of the Safety of Chronic FLOLAN Infusions Plus Conventional Therapy in Patients with Severe Primary Pulmonary Hypertension: A continuation Study".
- 20 May 92 Telephone call from FDA indicating that they would like a new
NDA submitted for treatment of PPH and that we should resubmit all data rather than cross reference the hemodialysis NDA.
- 06-04-92 Amended Dr. Nicholas Hill's Form FDA 1572 to include James
115 Klinger, M.D. as a subinvestigator to conduct clinical study 46, "A Multicenter, Open-Label, Randomized, Parallel Comparison of the Safety and Efficacy of Chronic FLOLAN Infusions Plus Conventional Therapy to Conventional Therapy alone in Patients with Severe Primary Pulmonary Hypertension: A Twelve-Week Study" and to register Bruce Brundage, M.D., Edgar Caldwell, M.D., Nicholas Hill, M.D., Srinivas Murali, M.D., and Barry Uretsky, M.D. as investigators to conduct clinical study 47, "A Multicenter, Open-Evaluation of the Safety of Chronic FLOLAN Infusions Plus Conventional Therapy in Patients with Severe Primary Pulmonary Hypertension: A Continuation Study".
- 06-11-92 In reference to meeting with FDA on August 19, 1991 and
116 telephone conversation on May 22, 1992, submitted the following genetic toxicology study reports:
- Evaluation of U-53,217A in the Salmonella/Microsome Test (Ames Assay) (Doc. 7200/81/7263/001).
- The Micronucleus Test with Prostacyclin (U-53,217A) (Doc. 0013/81/7263/002).
- Evaluation of U-53,217A (PGI₂) in the DNA Damage/Alkaline Elution Assay (Doc. 7263/80/7263/023).

- 07-28-92 Amended IND to register Edgar Caldwell, M.D. as an
117 investigator to conduct clinical study 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension" and Spencer Koerner, M.D., Cesar Keller, M.D., Michael McGoon, M.D. and Neil Ettinger, M.D. as investigators to conduct clinical study 47, "A Multicenter, Open-Evaluation of the Safety of Chronic FLOLAN Infusions Plus Conventional Therapy in Patients with Severe Primary Pulmonary Hypertension: A Continuation Study."
- 11 Aug 92 Telephone call from FDA, in response to our 10 Aug 92 telephone call, advising that summary data regarding preliminary survival results of our exercise tolerance study 46 be submitted for their review prior to any telephone discussion.
- 08-14-92 In reference to telephone conversation with FDA on August 11,
118 1992, submitted preliminary survival and exercise tolerance data which had recently become available from protocol 46, "A Multicenter, Open-Label, Randomized, Parallel, Controlled Comparison of FLOLAN plus Conventional Therapy to Conventional Therapy Alone in Patients with Severe, Primary Pulmonary Hypertension".
- 10 Aug 92 Telephone call to FDA informing them that our exercise tolerance study 46 was nearing completion and that preliminary survival results would be available by 13 Aug 92 and requesting their review of this data via telephone.
- 08-14-92 Letter from FDA to solicit our cooperation in establishing a single database containing ambulatory blood pressure measurements obtained from hypertensive patients receiving placebo in randomized clinical trials.
- 08-20-92 Amended IND to provide for clinical study 49, "A Multicenter,
119 Open Evaluation of the Safety of Chronic FLOLAN Infusions Plus Conventional Therapy in Patients with Severe Primary Pulmonary Hypertension: A Safety Study". Submitted an amended Form FDA 1572 for Dr. Neil Ettinger (previously submitted December 13, 1991 for protocol 46) who will be conducting this study. Study sites for this study will be limited to those investigators previously registered for protocol 46.
- 09-22-92 Amended IND to register Bennett deBoisBlanc, M.D. as an
121 investigator for clinical study 47 and Victor Tapson, M.D. as an investigator for clinical study 49.
- 09-25-92 Amended IND to provide for the use of a single vial formulation
120 of FLOLAN Injection, 0.5 mg and 1.5 mg, in clinical trials.
- 09-25-92 Letter to FDA to confirm the meeting to be held on October 6,
122 1992. As requested, submitted a complete statistical package for protocol 46, additional background information and an agenda for the meeting.
- 10-01-92 Telephone call to FDA to discuss an upcoming FLOLAN meeting.

- 6 Oct 92 Minutes of meeting with FDA to discuss 1) the clinical results from protocol 46, 2) the update on patients that were enrolled in protocol 45 and 47, 3) and their comments and suggestions regarding the TIND.
- 10-19-92 Letter from FDA requesting an Annual progress report.
- 10-20-92 Amended IND to register David Langleben, M.D., Montreal,
123 Quebec, Canada as an investigator to conduct clinical study 47.
- 10-26-92 Telephone conversations with FDA to request their participation
and in BW's Advisory Committee for the FLOLAN TIND; it was the
10-29-92 ir opinion that it would be more appropriate if they just reviewed
documents as they were provided under the IND.
- 10-29-92 Amended IND to register Syed Jafri, M.D. (who is replacing
124 Mihai Gheorghide, M.D.) as an investigator to conduct clinical
study 45 and to register the following investigators to conduct
clinical study 49:

David Badesch, M.D., FACP, FCCP
Bertron Groves, M.D.
William Clarke, M.D.
Stuart Rich, M.D.
Bruce Brundage, M.D.
Lewis Rubin, M.D.
Robyn Barst, M.D.
- 11-10-92 Amended IND to register Dr. David Langleben, Montreal,
125 Quebec, Canada as an investigator to conduct clinical study 49.
- 11-20-92 As agreed in meeting with FDA on October 6, 1992, submitted a
126 draft of the features required for the pump which will be used for
chronic infusion of FLOLAN under our treatment IND.
- 11-25-92 Telephone call to FDA to report the death of patient #01103 who
was being treated with FLOLAN under protocol 49; cause of
death uncertain at this time.
- 12-09-92 Telephone call from FDA to discuss our pump specifications to be
used for treatment of patients with PPH.
- 12-11-92 Telephone approval given to FDA authorizing them to refer to
our IND on behalf of Robyn Barst, M.D., of New York, NY to
support her IND41,252 for the treatment of a patient with
pulmonary hypertension.
- 12-15-92 Amended protocol 47, "A Multicenter, Open Evaluation of the
127 Safety of Chronic FLOLAN Infusions Plus Conventional Therapy
in Patients with Severe Primary Pulmonary Hypertension: A
Continuation Study", to provide for the use of the single vial
formulation which is supported by our Chemistry,
Manufacturing, and Control Amendment submitted
September 25, 1992.
- 12-30-92 As agreed in the meeting of October 6, 1992, submitted to FDA
128 for review a draft copy of the protocol for our planned Treatment
IND for FLOLAN.

- 01-07-93 Amended IND to register Adaani Frost, M.D., who is replacing
129 Cesar Keller, M.D. as principal investigator, to conduct clinical study 47 and Robert Bourge, M.D., Edgar Caldwell, M.D. and Adaani Frost, M.D. as investigators to conduct clinical study 49.
- 01-11-93 Telephone call to FDA regarding the status of their review of the
TIND protocol.
- 01-12-93 Letter to FDA authorizing them to refer to our IND on behalf of
130 Robyn Barst, M.D., of New York, NY to support her IND 41,252 for the treatment of a patient with pulmonary hypertension.
- 01-13-93 Telephone call to FDA with questions regarding the upcoming
PPH NDA.
- 01-14-93 Amended the following protocols to provide for the use of the
131 single vial formulation: 37, "A Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension" and 49, "A Multicenter, Open Evaluation of the Safety of Chronic FLOLAN Infusions Plus Conventional Therapy in Patients with Severe Primary Pulmonary Hypertension: A Safety Study".
- 01-15-93 Submitted to FDA copies of instructional videos ("Patient
132 Information" and "The Clean Routine") and of same transcripts which will be used in educating patients entered into the Treatment IND protocol.
- 01-26-93 Telephone call to FDA to determine the status of their review of
the proposed TIND protocol.
- 01-28-93 Telephone call from FDA to confirm that their review of the
proposed TIND protocol would be completed by January 29,
1993.
- 01-28-93 Telephone call to FDA to discuss the adverse events reported by
patients receiving the new single vial FLOLAN product.
- 02-01-93 Telephone call to FDA to follow-up on the review process of the
TIND protocol.
- 02-04-93 Telephone call to FDA to provide an updated status report on the
adverse experiences reported by eight of the patients receiving
the new single vial formulation.
- 02-11-93 Amended IND to register Christopher McGregor, M.B., Ch.B.,
133 FRCS as an investigator to conduct clinical study 21 and to register Bennett de Boisblanc, M.D. and Srinivas Murali, M.D., FACC, FACP/Barry Uretsky, M.D. (Co-principal investigators) as investigators to conduct clinical study 49.
- 02-16-93 Panafax received from FDA with comments on CMC submission
of September 25, 1992 providing for single vial formulation of
FLOLAN.

- 02-18-93
134 Amended IND to provide for revisions (to allow recording of hemodynamic measurements and vital signs for analysis of the efficacy and safety of the FLOLAN reformulation) to clinical study 49, "A Multicenter, Open Evaluation of the Safety of Chronic FLOLAN Infusion Plus Conventional Therapy in Patients with Severe Primary Pulmonary Hypertension: A Safety Study".
- 02-18-93 Letter received from FDA advising that the proposed treatment IND protocol is acceptable but that the study may not be initiated until a satisfactory response to chemistry issues raised in their February 16, 1993 letter relevant to safety is submitted. are resolved. A copy of this letter was panafaxed to us on February 17, 1993.
- 03-05-93
135 In reference to telephone conversations with FDA on January 28 and February 4, 1993 and their letter of February 18, 1993, submitted to FDA a copy of our letter sent to investigators which summarized our findings documenting that the recent problems in some patients switched to the new formulation were not due to the new formulation, but were due to a variety of other factors which are preventable.
- 03-18-93
136 Amended IND to register Michael McGoon, M.D., Nicholas Hill, M.D. and Spencer Koerner, M.D. as investigators to conduct clinical study 49.
- 04-05-93 Letter from FDA requesting an IND annual report.
- 04-16-93
137 In reference to FDA's request of April 5, 1993, submitted an Annual Report for the period of April 1, 1991 through March 31, 1992 and submitted the revised Investigator's Brochure. Informed FDA of our intention in the future to submit INDs 16,459 and 38,609 as a combined IND Annual Report.
- 16 Apr 93 Telephone call to FDA to report the death of patient #02104 who was being treated under protocol 49 by Lewis Rubin, M.D.
- 10 Jun 93
138 Amended IND to register Cesar Keller, M.D. as an investigator to conduct clinical study 49.
- 17 Jun 93
139 Letter to FDA in reference to telephone conversation of 16 Apr 93, submitted an IND Safety Report for patient #02104 who experienced ventricular tachycardia and expired while being treated under protocol 49, "A Multicenter, Open Evaluation of the Safety of Chronic FLOLAN Infusions Plus Conventional Therapy in Patients with Primary Pulmonary Hypertension: A Safety Study ", being conducted by Dr. Lewis Rubin.
- 18 Jun 93 Telephone call to FDA to inform them of the termination of CHF study 48 by the DSMB; we assured FDA that this would not effect our plans for the PPH NDA.
- 28 Jun 93 Telephone call from FDA requesting the emergency treatment of a patient with severe PPH secondary to Lupus erythematosus under an investigator IND.

- 29 Jun 93 Telephone call to FDA in response to their request of 28 Jun 93 regarding our willingness to provide FLOLAN to a patient with severe PPH secondary to Lupus erythematosus under an investigator IND.
- 7 Jul 93 Letter to FDA in response to their letter of 16 Feb 93 with
140 questions concerning the chemistry, manufacturing and controls data for the new single vial formulation in our amendment dated 25 Sep 92. Notified FDA that development of the single vial formulation has been discontinued.
- 9 Jul 93 Letter to FDA amending the IND to provide for Greenville, NC
141 as an additional manufacturing site of the Sterile Diluent for FLOLAN for use in clinical trials.
- 9 Jul 93 Telephone call to FDA to report that Patient #15105 experienced
acute pulmonary congestion and Patient #01102 experienced temporary blurry vision associated with a headache while enrolled under protocol 49.
- 19 Jul 93 Amended IND to provide for clinical study 50, "A Multicenter,
143 Open, Compassionate-Use Protocol to Provide Chronic FLOLAN Infusions Plus Conventional Therapy to Patients with Severe Primary Pulmonary Hypertension", to be conducted by Bertron Groves, M.D. and David Badesch, M.D.
- 20 Jul 93 Amended IND to register Henry Mizgala, M.D., FRCP,
144 Vancouver, British Columbia, Canada as an investigator to conduct clinical study 49.
- 24 Aug 93 Amended IND to register Stuart Rich, M.D., Neil Ettinger, M.D.
145 and Adaani Frost, M.D. as investigators to conduct clinical study 50.
- 27 Aug 93 Letter to FDA submitting an Annual Report for the period 1 Apr
146 92 through 31 March 93.
- 2 Sep 93 Amended IND to register Robert Bourge, M.D. as an investigator
147 to conduct clinical study 21 and to register Spencer Koerner, M.D. as an investigator to conduct clinical study 50.
- 9 Sep 93 Letter to FDA, in reference to telephone conversation on 2 Sep 93,
148 requesting a meeting with FDA during the week of 18 October 93 to discuss the NDA filing strategy and to address the manufacturing and controls data intended to support the filing.
- 9 Sep 93 Telephone call to FDA to request a meeting with FDA to discuss
Chemistry, Manufacturing and Control issues regarding our planned NDA and to update them on our plans regarding the single vial formulation, NDA strategy and the Compassionate Use Trial v. Treatment IND.
- 23 Sep 93 Amended IND to register William Clarke, M.D. as an investigator
149 to conduct clinical study 50.
- 23 Sep 93 Telephone call from FDA to advise that the meeting we requested
on 9 Sep 93 to discuss CMC issues in connection with our proposed NDA has been scheduled for 15 Nov 93.

- 30 Sep 93 150 Amended IND to provide for revisions (amendment #1, to allow those patients in continuation protocols 37, 47, and 49 to be enrolled in protocol 50) to clinical study 50. .
- 8 Oct 93 Letter from The Upjohn Company along with a copy of their letter from FDA granting them approval to export epoprostenol sodium to Spain.
- 15 Oct 93 151 Amended IND to register Robyn Barst, M.D., Cesar Keller, M.D., and Edgar Caldwell, M.D. as investigators to conduct clinical study 50.
- 25 Oct 93 Telephone call to FDA to confirm the meeting scheduled for 15 Nov 93 and to inform them that background data for the meeting would be sent this week.
- 27 Oct 93 152 Amended IND to register Michael McGoon, M.D. as an investigator to conduct clinical study 50.
- 29 Oct 93 153 Letter to FDA, in reference to telephone conversations on 9 and 23 Sep 93 and 25 Oct 93 concerning our request for a meeting to discuss our upcoming NDA, submitting nine copies of summary information of the chemistry, manufacturing, and controls data for pre-meeting review by the attendees and a revised agenda for the 15 Nov 93 meeting.
- 19 Nov 93 154 Amended IND to register Bruce Brundage, M.D. and Lewis Rubin, M.D. as investigators to conduct clinical study 50.
- 23 Nov 93 155 Letter to FDA submitting minutes from the 15 Nov 93 meeting and a full copy of the information presented.
- 29 Nov 93 156 Letter to FDA submitting an IND Safety Report for patient #89 who developed severe hypotension and died after discontinuing FLOLAN while enrolled in clinical study 21, being conducted by Dr. Robyn Barst; also submitted a copy of the Dear Dr. letter addressing this experience.
- 10 Dec 93 157 Letter to FDA submitting stability data and data supporting the reconstitution and dilution studies to be evaluated for their acceptability for filing in the NDA as requested in the meeting held on 15 Nov 93.
- 12 Jan 94 158 Amended IND to register Nicholas Hill, M.D. and Robert Bourge, M.D. as investigators to conduct clinical study 50.
- 14 Jan 94 159 Letter to FDA, in reference to telephone conversation of 7 Jan 94, submitting an IND Safety Report for patient #09205, who experienced hypotension while enrolled in study 50, being conducted by Dr. Bruce Brundage. Also submitted a copy of the Dear Dr. letter pertaining to this incident.
- 14 Jan 94 160 Amended IND to provide for revisions (amendment #2) to clinical study 50.
- 1 Feb 94 Letter from FDA with requests regarding our 10 December 93 submission which provided stability data and data supporting the reconstitution and dilution studies.

- 9 Feb 94 Amended IND to register Srinivas Murali, M.D. and Barry
161 Uretsky, M.D. as co-principal investigators to conduct clinical
study 50.
- 17 Feb 94 Letter to FDA submitting our response to comments contained in
162 their 1 February 1994 letter regarding our 10 December 93
submission.
- 28 Feb 94 Letter to FDA submitting the final report for an ongoing
163 reconstitution study in response to FDA's comments in their 1
Feb 94 letter regarding our amendment of 10 Dec 93 providing
stability data and data supporting the reconstitution and dilution
studies. This data was also included in the NDA submitted on 28
Feb 94.
- 1 Mar 94 Amended IND to register Jay Fricker, M.D. as an investigator to
164 conduct clinical study 50.
- 3 Mar 94 Letter to FDA submitting a "Summary of the Pharmaceuticals,
165 Clinical Pharmacology and Preclinical Pharmacology" on the
single-vial formulation used in clinical trials for treating patients
with PPH. This summary was incorporated by reference to NDA
20-444 as agreed in our meeting with FDA on 15 Nov 93.
- 31 Mar 94 Letter to FDA, in reference to telephone conversation of 22 Mar
166 94, submitting IND Safety Reports for two patients who were
enrolled in protocol 50: Patient #17111 who experienced a
cardiac arrest and subsequently expired while being treated by
Dr. Adaani Frost; and Patient #01011 who experienced
hemothorax and expired while being treated by Dr. Robyn Barst.
- 31 Mar 94 Amended IND to register David Langleben, M.D., Montreal,
167 Quebec, Canada as an investigator to conduct clinical study 50.
- 6 Apr 94 Amended IND to register Bennett deBoisblanc, M.D. as an
168 investigator to conduct clinical study 50.
- 11 Apr 94 Amended IND to register Victor Tapson, M.D. as an investigator
169 to conduct clinical study 50.
- 11 Apr 94 Letter to FDA submitting an adverse event report involving the
CADD-1 portable infusion pump as a follow-up to our IND
Safety Report submitted on 31 Mar 94.
- 22 Apr 94 Telephone call from FDA to inform us of a request from Dr.
Robert Baker to treat a patient with pulmonary hypertension.
- 26 Apr 94 Amended IND to register David Badesch, M.D. as a co-principal
170 investigator with Bertron Groves, M.D. to conduct clinical study
21.
- 29 Apr 94 Letter to FDA, submitting an IND Safety Report for patient
171 #16201 who experienced intrapulmonary hemorrhage and died
while enrolled in protocol 50, being conducted by Dr. David
Langleben.
- 3 May 94 Amended IND to register David Ostrow, M.D., Vancouver,
172 British Columbia, Canada to conduct clinical study 50.

- 11 May 94 173 Letter to FDA, in reference to telephone conversation of 29 Apr 94, submitting an IND Safety Report for patient #01113 who died suddenly while enrolled in study 50, being conducted by Dr. Robyn Barst.
- 25 May 94 Telephone call from FDA regarding a Congressional inquiry concerning a request which BW had denied for treatment of a patient (CH) with PPH due to the potential risks to the patient being greater than the possible benefits. Two conference calls on this same date resulted in BW agreeing to confer with the investigator, Stuart Rich, M.D. following a further evaluation of the patient's condition.
- 1 Jun 94 174 Letter to FDA, in reference to telephone conversation on 24 May 94, submitting an IND Safety Report for patient #10202 who experienced a transient ischemic attack while enrolled in study 50, being conducted by Nicholas Hill, M.D. Also, as requested, submitted the pulmonary artery pressure results at catheterization and a copy of the letter to investigators addressing this experience.
- 11 Jul 94 175 Letter to FDA submitting an Annual Report for the period 1 Apr 93 through 31 Mar 94.
- 5 Aug 94 176 Letter to FDA submitting an IND Safety Report for patient #04204 who experienced a migraine headache while enrolled in clinical study 50, being conducted by Edgar Caldwell, M.D.
- 1 Nov 94 Telephone call to FDA to discuss the Dr. Stuart Rich's (investigator) request to treat a female patient with PPH, who is pregnant, under protocol 50. FDA approved the treatment exception providing that an IRB approval and a signed informed consent form were required from the patient.
- 8 Nov 94 Telephone call to FDA to provide a summary on a patient who experienced bradycardia, syncope and died while being treated on a compassionate basis in Japan.
- 23 Nov 94 177 Letter to FDA, in reference to telephone conversation of 8 Nov 94, submitting an IND Safety Report for patient N-Y who experienced fatal bradycardia while being treated on a compassionate basis in Japan.
- 1 Dec 94 178 Letter to FDA submitting an IND Safety Report for patient #14201 who experienced post operative bleeding while enrolled in clinical study 50, being conducted by William Clarke, M.D.
- 17 Jan 95 179 Letter to FDA submitting an IND Safety Report for patient #17112 who experienced suspected anaphylaxis to streptokinase and subsequently died while enrolled in protocol 50, being conducted by Dr. Adaani Frost.
- 31 Mar 95 180 Letter to FDA, in reference to the IND Safety Report submitted on 17 Jan 95, submitting the provisional autopsy report for patient #17112 who experienced suspected anaphylaxis to streptokinase and subsequently died while enrolled in protocol 50.

- 11 Apr 95 Letter to FDA, in reference to telephone conversation of 4 Apr 95,
181 submitting an IND Safety Report for patient #13205 who developed a hemopneumothorax during Hickman catheter replacement due to acinetobacter infection and subsequently died while enrolled under protocol 50, being conducted by Dr. Srinivas Murali. In addition, submitted an IND Safety Report for patient #17202 who developed a hemothorax during Hickman catheter replacement to alleviate recurrent line while enrolled under protocol 50, being conducted by Dr. Adaani Frost.
- 8 May 95 Letter to FDA submitting an IND Safety Report for patient
182 #07201 who experienced bacteremia with related immune-complex glomerulonephritis while enrolled in protocol 50, being conducted by David Badesch, M.D.
- 10 May 95 Telephone call to FDA to inform of the death of patient #09244
who was being treated under protocol 50.
- 17 May 95 Letter to FDA submitting an IND Safety Report for patient
183 #15211 who experienced pulmonary edema while enrolled in protocol 50, being conducted by Michael McGoon, M.D.
- 18 May 95 Letter to FDA submitting an IND Safety Report for patient
184 #09244 who experienced hypotension and subsequently expired while enrolled in protocol 50, being conducted by Dr. Bruce Brundage.
- 16 Jun 95 Telephone call to FDA to report an adverse event for a patient
enrolled under protocol 21.
- 19 Jun 95 Letter to FDA submitting an IND Safety Report for patient
185 #07006 who bled intermittently from the catheter insertion site, over a three day period following Hickman catheter replacement, while enrolled in protocol 50, being conducted by Dr. David Badesch.
- 22 Jun 95 Telephone call to FDA to report that patient #02218 experienced
endocarditis and subsequently died while being treated under protocol 050.
- 27 Jun 95 Letter to FDA in reference to telephone conversations of 16 and
186 22 Jun 95, submitting an IND Safety Report for patient #82 who experienced increased shunting with hypoxemia while enrolled in protocol 21, being conducted by Dr. Berton Groves and for patient #02218 who experienced endocarditis while enrolled in protocol 50, being conducted by Dr. Lewis Rubin.
- 28 Jun 95 Amended IND to register Brad Warner, M.D. as an investigator to
187 conduct clinical study 21.
- 14 Aug 95 Letter to FDA, in reference to our 15 Oct 95 submission
188 registering Dr. Robyn Barst of Columbia Presbyterian Babies Hospital for protocol 50, informing them of the addition of Quantum Health Resources, 150 Lake Dr., Wexford PA as a Contract Research Organization for distribution of CTM and related supplies to Columbia Presbyterian Babies Hospital.

- 25 Feb 94 Submitted user fee for this NDA.
- 28 Feb 94 Submitted an Original New Drug Application to provide for use in the treatment of primary pulmonary hypertension (PPH).
- 28 Feb 94 Submitted methods validation for the original NDA.
- 2 Mar 94 Telephone conversation with FDA to discuss the biopharmaceutics/pharmacokinetics reviewer's request for stereospecific pharmacokinetic information.
- 8 Mar 94 Letter from FDA acknowledging receipt of our NDA submitted; if accepted, 28 February 1994; stated that 29 April 1994 will be the filing date.
- 11 Mar 94 Submitted a User Fee Cover Sheet to complete User Fee requirements for our primary pulmonary hypertension NDA submitted 28 February 1994.
- 30 Mar 94 Telephone call from FDA to request diskettes containing the SAS listings for pivotal studies 35/36 and 46.
- 31 Mar 94 Telephone call from FDA to request 1) Normal Values for pulmonary hemodynamics from two hospitals used in the pivotal trials (studies 35/36/46); 2) a definition of Wood Units as a measure of vascular resistance; 3) a clear description of the randomization procedure used in Study 46.
- 6 Apr 94 As requested by FDA in 30 March 1994 telephone conversation, submitted diskettes containing SAS listings for studies 35/36 and 46 along with hard copies of the annotated case report for each study and the phase/phase sequence/subset list.
- 7 Apr 94 In response to FDA telephone requests on 31 March 1994, submitted, 1) normal values for pulmonary hemodynamics from two hospitals used in the pivotal trials (studies 35/36/46); 2) a definition of Wood Units as a measure of vascular resistance; 3) a clear description of the randomization procedure used in Study 46.
- 8 Apr 94 Telephone call from FDA to request a meeting on 14 April 1994, to discuss FDA concerns, re: the Human Pharmacokinetics, Chemistry and Microbiology sections of our NDA.
- 14 Apr 94 Minutes of meeting with FDA to discuss NDA filing issues.
- 15 Apr 94 Telephone call to FDA to provide information regarding patients in Study 46 who received "Dartford" manufactured drug vx. "Upjohn" manufactured drug.
- 20 Apr 94 As requested by FDA in 14 April 1994 meeting, submitted "A Summary of the Use of Epoprostenol Sodium Synthesized by Wellcome Research Laboratories (Dartford, England) in Study 46" (Document No. THZZ/94/0185).
- 21 Apr 94 Received by panafax from FDA, a request for additional information needed for the NDA microbiology review.
- 22 Apr 94 Telephone call from FDA to request 1) a copy of the "Adaptive Randomization Computer Program" used for Study 46, and 2) patient identifiers used for the randomization code.

- 22 Apr 94 As requested in meeting with FDA on 14 April 1994, submitted "Rationale for Waiver of Human Pharmacokinetics and Bioavailability Requirements for FLOLAN (epoprostenol sodium) for Injection".
- 25 Apr 94 As requested by FDA in 22 April 1994 telephone call, submitted a copy of the "Adaptive Randomization Computer Program" used for the randomization procedure in Study 46; a copy of this randomization procedure was previously submitted on 11 November 1991 to our IND 16,459.
- 29 Apr 94 Submitted a summary of a telephone conference call with FDA on 20 April 1994, concerning our proposed stability data package and the microbiological review of our NDA.
- 29 Apr 94 Submitted responses to microbiology questions contained in the FDA letter received by telefacsimile on 21 February 1994; a field copy was also provided to the local FDA district office.
- 29 Apr 94 Telephone call to FDA in which the FDA confirmed that the NDA was accepted for filing this date.
- 4 May 94 Telephone call from FDA to request 1) an electronic and hard copy of the actual randomization program; 2) procedure for implementation, and 3) randomization code.
- 6 May 94 Letter from FDA with recommendations and requests concerning the environmental assessment submitted 28 February 1994.
- 6 May 94 As requested by FDA in 4 May 1994 telephone conversation, submitted diskettes containing the source code and the compiled code for the randomization program, and provided documentation describing the randomization procedure.
- 9 May 94 Letter from FDA requesting clarification on points relating to the NDA statistical review.
- 16 May 94 Telephone call from FDA's Compliance Division to request information to use in preparing for the clinical inspections in connection with our NDA.
- 17 May 94 Telephone call from FDA's Compliance Division requesting additional information for clinical inspections.
- 17 May 94 As requested in 16 May 1994 telephone conversation, panafaxed to FDA tables which provide a list of investigators for NDA studies 35, 36 and 46.
- 14 Apr 94 Minutes of meeting with FDA concerning the NDA Manufacturing and Control, including Microbiology (Item 3), and Human Pharmacokinetics (Item 6) data.
- 27 May 94 Submitted response to FDA letter of 9 May 1994, concerning the NDA statistical review.
- 27 May 94 As requested by FDA in 16 and 17 May 1994 telephone conversations, submitted information for FDA's use in preparing for clinical inspections.
- 2 Jun 94 Telephone call to FDA to obtain clarification to questions 1.c and 3.e, in the 6 May 1994 environmental assessment deficiency letter.

- 7 Jul 94 Letter to FDA providing authorization from The Upjohn Company for B.W. Co. to reference their Drug Master File No. 5319.
- 27 Jul 94 Submitted the four month safety update.
- 28 Jul 94 As requested by FDA in 26 July 1994 telephone call, submitted case report forms for patients in Study 46 who died or were transplanted.
- 1 Aug 94 Letter from FDA questions and requests concerning the NDA review.
- 3 Aug 94 Telephone call from FDA to request data tabulations of hemodynamic values collected in Study 46 for each study site and all patients.
- 5 Aug 94 As requested by FDA in 3 August 1994 telephone call, submitted data tabulations of hemodynamic values for each study site and all patients in study 46.
- 23 Aug 94 Submitted responses to FDA questions in their 1 August 1994 letter, re: NDA review.
- 14 Sep 94 Submitted a revised Environmental Assessment and our response to FDA comments contained in 6 May 1994 letter.
- 16 Sep 94 Submitted updated stability data as committed to 20 April 1994 telephone conference with FDA and our 29 April 1994 submission.
- 16 Sep 94 Telephone call from FDA's Compliance Division to request, after discussions with the Medical Officer, additional analysis of adverse drug reactions which occurred during the dose-ranging segment of Study 46.
- 23 Sep 94 Letter from FDA requesting information pertinent to the NDA clinical and statistical review.
- 23 Sep 94 Letter from FDA stating that our 14 September 1994 Environmental Assessment submission is considered a major amendment and that 60 additional days will be required to complete the NDA review.
- 23 Sep 94 Telephone call from FDA to review the status of our responses to their requests, re: stability update, and the Upjohn response to Drug Master File deficiencies.
- 28 Sep 94 Telephone call to FDA to obtain clarification of their requests in their 23 September 1994 letter, re: clinical and statistical information pertinent to the NDA review.
- 3 Oct 94 Telephone call from FDA to provide comments concerning the revised Environmental Assessment submitted 14 September 1994.
- 4 Oct 94 As requested by FDA in 16 September 1994 telephone conversation, submitted additional analyses of adverse drug reactions which occurred in the dose-ranging segment of study 46.

- 6 Oct 94 As requested by FDA in 3 October 1994 telephone conversation, provided replacement pages 1-4 and 7 of the Environmental Assessment submitted 14 September 1994.
- 17 Oct 94 Received by panafax from FDA, letter requesting additional or clarifying information to support the sterilization process validation information portion of our NDA.
- 20 Oct 94 Telephone call from FDA to advise that Flolan had been placed on the agenda for the 23-24 February 1995 Cardio-Renal Advisory Committee.
- 27 Oct 94 Letter to FDA requesting a meeting to discuss the Flolan Advisory Committee meeting scheduled for 23-24 February 1995.
- 28 Oct 94 As requested by FDA in 23 September 1994 telephone call, submitted additional clinical and statistical information for the NDA review.
- 1 Nov 94 Letter from FDA stating that the additional NDA clinical and statistical information submitted 28 October 1994, is considered a major amendment and the regulatory due date has been extended to 25 March 1995; the due date under the Prescription User Fee Act of 1992 remains 27 February 1995.
- 10 Nov 94 Letter from FDA stating that the medical review of our NDA is complete and contact will be made to arrange a meeting to discuss the application and the Advisory Committee meeting which is scheduled for February. (Copies of medical and statistical reviews attached.)
- 10 Nov 94 Telephone call from FDA to report that BW Co. will be receiving a letter with review comments on the clinical section of the NDA, and they wish to schedule a meeting to discuss the comments.
- 18 Nov 94 Telephone conversation with FDA to discuss the NDA review; agreed that a meeting between BW and FDA should be held prior to the advisory committee meeting.
- 23 Nov 94 Letter to FDA confirming our 5 December 1994 meeting with the Division of Gastrointestinal and Coagulation; also included a proposed agenda and the list of BW attendees.
- 6 Dec 94 Letter from FDA requesting copies of Case Report Forms for the patients who were transplanted or withdrew from Study 49 and Study 50.
- 8 Dec 94 Telephone call from FDA to relay information that should be included in BW's package to the Advisory Committee, and what our presentations should be.
- 8 Dec 94 Telephone call from FDA to state that the diskette (dated October 1994) containing survival data for Studies 35/36, 37, 46 and 47, and the NIH registry is not readable; requested a new diskette in SAS PC format with additional information for Study 35/36, and detailed instructions on how to read the diskette.
- 12 Dec 94 As agreed in meeting with FDA on 5 December 1994, submitted responses to questions/comments resulting from the Medical Review of our NDA.

- 13 Dec 94 Telephone conversations with FDA Statistician concerning his request for a new diskett, in SAS PC format, containing survival data.
- 14 Dec 94 Letter from FDA with comments and requests concerning the NDA statistical review.
- 23 Dec 94 As requested by FDA in 6 December 1994 letter, submitted case report forms for patients who were transplanted or withdrew from Study 49 and Study 50.
- 23 Dec 94 Received by panafax, a copy of a letter The Upjohn Company received from the FDA advising that the Detroit District has recommended that the Center for Drug Evaluation and Research to approve the NDA.
- 5 Jan 95 Panafax received from FDA with additional statistical request for Studies 49 and 50.
- 11 Jan 95 Telephone call from FDA (11th) to request a meeting with BW on
and 30 January to demonstrate the randomization program used in
Study 46 of the NDA clinical trials., follow-up telephone call
12 Jan 95 from FDA (12th) to state that the Division Director approves of
copies of the summary package being forwarded to the Advisory
Committee prior to his comments.
- 11 Jan 95 Letter from FDA requesting additional information for studies 49
and 50, to complete the NDA statistical review.
- 12 Jan 95 FDA Medical Reviewer comments of our 120-day Safety Update.
- 12 Jan 95 As discussed, submitted a draft of our Advisory Committee
summary to the FDA for review and comment.
- 19 Jan 95 Received by panafax from FDA a letter requesting information
pertinent to the NDA statistical review.
- 23 Jan 95 Telephone call from FDA to advise that review of our proposed
Advisory Committee package has been completed.
- 24 Jan 95 Submitted response to FDA's 17 January 1995 request for
information to complete the NDA statistical review.
- 25 Jan 95 Submitted a replacement diskette for diskette #1, entitled
"Randomization program used in FLOLAN Study 46 with an
empty data file, PAT46.DBF", originally submitted 24 January
1995 in response to their 17 January 1995 request.
- 25 Jan 95 As requested, submitted to FDA's District Office a copy of the
original randomization program used in Study 46 and the
version of program in which the study was written.
- 25 Jan 95 Telephone call to FDA to advise that requested diskettes for the
randomization program has been forwarded, and BW Co. has
received FDA comments concerning review of the Advisory
Committee package.
- 27 Jan 95 Submitted response to FDA comments in letter of 17 October
1994, re: sterilization process validation provided in our original
NDA. (A field copy was provided to the local FDA District
Office.)

31 Jan 95 Telephone call to FDA to confirm their use of PPH data replacement diskettes submitted 25 January 1995.

1 Feb 95 Received by panafax from FDA Advisory Committee draft questions concerning NDA Studies 35/36 and 46.

1 Feb 95 Telephone call to FDA in response to FDA questions in 31 January 1995 telephone conversation, re: diskette version of randomization program.

2 Feb 95 Submitted by reference, update from Upjohn's Drug Master File 6065 data for Epoprostenol Sodium to our NDA.

3 Feb 95 Panafaxed to FDA, our comments to their draft questions received 1 February 1995.

3 Feb 95 Telephone call from FDA to request a diskette copy of our package insert in Word Perfect format.

6 Feb 95 As requested by FDA in 3 February 1995 telephone call, provided a diskette copy in Word Perfect format of the package insert identical to the insert submitted with the New Drug Application.

6 Feb 95 Provided a summary package to FDA to use in preparing for the 23 February 1995 Cardio-Renal Advisory Committee meeting.

9 Feb 95 Copies of final medical and statistical reviews picked up at FDA.

13 Feb 95 Telephone call to FDA to request meetings to resolve their concerns regarding validation of the randomization program used in Study 46, and to correct factual errors identified in the NDA review.

14 Feb 95 Telephone call from FDA to advise that a meeting is scheduled for 16 February 1995 to discuss issues related to the Advisory Committee.

14 Feb 95 Follow-up telephone call from FDA to request the purpose for the 16 February 1995 meeting between FDA/BW Co. scheduled prior to the Advisory Meeting.

14 Feb 95 Telephone call to FDA to request a meeting with the statisticians to discuss the adaptive randomization program.

15 Feb 95 Telephone call from FDA to postpone the 16 February 1995 meeting until 21 February 1995.

15 Feb 95 Telephone call from FDA to advise that a Statistical Meeting is scheduled for 21 February 1995, following the scheduled FDA/BW Co. meeting.

15 Feb 95 Return telephone call to FDA to discuss a meeting time with the statisticians to discuss the NDA.

23 Feb 95 Copy of questions presented to the Cardiovascular and Renal
and Drugs Advisory Committee members and their responses
discussed during their 23 and 24 February 1995 meeting; also
24 Feb 95 included are materials presented to the Advisory Committee
members to support approval for the NDA.

- 6 Mar 95 Telephone call from FDA to request a background history of Patient 01012 entered into Study 46, and to confirm that BW is preparing a revised package insert, based on Advisory Committee comments; also discussed the status of the Chemistry Manufacturing and Controls, Microbiology, Pharmacology and Biopharmaceutics review.
- 6 Mar 95 Letter from FDA stating that the NDA chemistry, manufacturing and controls review is completed; requested explanations for questions concerning the drug substance and product.
- 13 Mar 95 Telephone conversation with FDA concerning the status of a PAI inspection at the Greenville facility for the manufacture of the Sterile Diluent.
- 16 Mar 95 Telephone call from FDA concerning the status of Patient 01012 background history and the revised package insert, requested in their 6 March 1995 telephone call.
- 17 Mar 95 Telephone call to FDA to advise that the background history of Patient 01012 (Study 46), and the revised labeling is in preparation.
- 20 Mar 95 Submitted revised draft package insert with revisions based upon the Advisory Committee recommendations.
- 21 Mar 95 As requested by FDA in 6 March 1995 conversation, submitted the clinical case history of Patient 01012 entered in Study 46.
- 31 Mar 95 Telephone call from FDA to advise that a second deficiency letter with additional comments/questions has been forwarded to Upjohn on their Drug Master File.
- 10 Apr 95 Submitted response to the Chemistry, Manufacturing, and Controls comments in the 6 March 1995 letter from FDA.
- 12 Apr 95 As discussed with FDA in 12 April 1995 telephone conversation, submitted final printed container labeling for FLOLAN and the accompanying Sterile Diluent.
- 12 Apr 95 Telephone call from FDA to request data to support the acute and chronic dosing proposed in our labeling for children.
- 13 Apr 95 As requested in 12 April 1995 telephone conversation with FDA, submitted the demographic information (from clinical studies contained in our NDA) to support pediatric use of FLOLAN.
- 14 Apr 95 Minutes of a meeting with FDA to discuss specific items of the NDA.
- 20 Apr 95 As discussed in a 17 April 1995 telephone conversation with FDA, submitted a summary of mean change from baseline to maximum tolerated dose for each hemodynamic parameter in patients less than 16 years old (n=60) and greater than or equal to 16 years old (n=314) during acute dose ranging with FLOLAN.
- 20 Apr 95 As agreed in telephone conversation with FDA on 17 April 1995, submitted the corrected tables for a programming error in Study 46.

- 22 Apr 95 Received by panafax from FDA a draft letter stating that the product microbiological quality and sterility assurance information is complete and the sterilization process validation; requested a post-approval commitment.
- 24 Apr 95 Telephone call from FDA to request a post NDA commitment regarding the Microbiology section.
- 27 Apr 95 Telephone conference call with FDA in which the FDA requested a commitment from BW Co. to provide details of how the filter validation studies were conducted.
- 27 Apr 95 Provided by panafax to FDA a note stating that the patent information previously provided for our NDA should have included the 25 and 50 mg tablet strength, in addition to the 100, 150, 200 and 250 mg.
- 28 Apr 95 Letter from FDA listing their requests for filter validation information as discussed in the 27 April 1995 telephone conversation.
- 28 Apr 95 Letter from FDA with recommendations and requests pertaining to the NDA chemistry review.
- 28 Apr 95 As discussed with FDA in 12 April 1995 telephone conversation, submitted additional information in support of the pediatric indication for our NDA.
- 1 May 95 Telephone call from FDA to inform us that the NDA package had been sent to Dr. Temple's Office for his review and approval.
- 3 May 95 As discussed with FDA in 24 and 27 April 1995 telephone conversations, letter to FDA with a post-approval commitment to provide additional information concerning sterilization process validation information.
- 4 May 95 Letter from FDA requesting current NDA safety information.
- 5 May 95 Telephone call from FDA to request a safety update to our NDA.
- 9 May 95 Letter from FDA stating that our NDA is approvable; requested final printed labeling and our response to their 28 April 1995 request for additional chemistry information.
- 12 May 95 Letter to FDA stating that a response to their comments in the 9 May 1995 approvable letter will be submitted in the near future.
- 22 May 95 Submitted a revised draft package insert in response to the 9 May 1995 NDA approvable letter.
- 31 May 95 Telephone call from FDA to request the number of pediatric patients in the acute dose studies who had Primary Pulmonary Hypertension, and data to support the chronic benefit.
- 2 Jun 95 As requested, provided FDA with the number of patients who had primary pulmonary hypertension.
- 6 Jun 95 In response to the 9 May 1995 approvable letter, submitted final printed container labeling for FLOLAN and the accompanying Sterile Diluent.

- 7 Jun 95 In response to FDA telephone requested of 30 May 1995, submitted a copy of Protocol 46 and Protocol 47.
- 9 Jun 95 Submitted additional comments concerning placement of lot numbers and expiration dates on final printed container labeling.
- 9 Jun 95 Letter to FDA providing our response to their comments contained in 28 April 1995 letter.
- 22 Jun 95 Submitted the approval safety update.
- 7 Jul 95 Submitted the user fee payment to cover the balance of the application fee for review.
- 11 Jul 95 Telephone call from FDA requesting that we submit a new Methods Validation package to incorporate the revisions which have occurred as a result of the CMC review of the pending NDA.

24 Jul 95 Letter to FDA incorporating amended DRIF from CIP/1111 to NDA

27 Jul 95 Internal communication Dr. Badolochi's request to tx pt c CREST

9 Aug 95 Letter from FDA acknowledging receipt of 7/24/95 letter

23 Aug 95 Letter to FDA confirming data in Table CHD 2.1
changes shown in last comment

9 Sep 95 - to FDA from 11 Sep 1995 re: 11 Sep 1995 letter to FDA
15 Sep 95 - Temple received completed letter
20 Sep 95 approval

28 Sep 95 Letter to FDA acknowledging receipt of 11 Sep 1995 letter

- 7 Jun 95 In response to FDA telephone requested of 30 May 1995, submitted a copy of Protocol 46 and Protocol 47.
- 9 Jun 95 Submitted additional comments concerning placement of lot numbers and expiration dates on final printed container labeling.
- 9 Jun 95 Letter to FDA providing our response to their comments contained in 28 April 1995 letter.
- 22 Jun 95 Submitted the approval safety update.
- 7 Jul 95 Submitted the user fee payment to cover the balance of the application fee for review.
- 11 Jul 95 Telephone call from FDA requesting that we submit a new Methods Validation package to incorporate the revisions which have occurred as a result of the CMC review of the pending NDA.

24 Jul 95 Letter to FDA incorporating amended DMF from UP John to NDA

27 Jul 95 Internal communication Dr Balesch's request to tx pt ECREST

9 Aug 95 Letter from FDA acknowledging receipt of 7/24/95 letter

23 Aug 95 Letter to FDA confirming data in Table (HD ~ chronic Admin) are correct.

9 Sep 95 - tel call from CSC that NDA signed by Dr Fresh & deliv to Temple

all to FDA

15 Sep 95 - Temple had not completed review

20 Sep 95 approval

28 Sep 95 Letter to FDA amending Method 2 Validation
proof

EXHIBIT 8

PATENT TERM EXTENSION CALCULATIONS
for U.S. PATENT 4,883,812

EXHIBIT 8 U.S. 4,883,812

Patent Term Extension Calculations

IND Effective Date: 6/29/79

6/29/79 - 11/27/89

Patent Issue Date: 11/28/89

11/28/89 - 12/31/89 = 33

1/1/90 - 12/31/90 = 365

1/1/91 - 12/31/91 = 365

1/1/92 - 12/31/92 = 366

1/1/93 - 12/31/93 = 365

1/1/94 - 2/27/94 = 58

1552 x 0.5 = 776 days

NDA Submission Date: 2/28/94

2/28/94 - 12/31/94 = 307

1/1/95 - 9/20/95 = 263

570 x 1 = 570 days

NDA Approval Date: 9/20/95

776 + 570 = 1346 days

Patent Term Extension + Expiration as terminally disclaimed against U.S. 4,539,333

**Expiration as terminally
disclaimed against U.S. 4,539,333:** 9/3/02

9/3/02 - 12/31/02 = 119

1/1/03 - 12/31/03 = 365

1/1/04 - 12/31/04 = 366

1/1/05 - 12/31/05 = 365

1/1/06 - 5/11/06 = 131

1346 days

**Expiration as terminally
disclaimed against U.S. 4,539,333
+ 1346 day Patent Term Extension:** 5/11/06

14 yr Cap from NDA Approval Date

NDA Approval Date: 9/20/95

9/20/95 + 14 yrs = 9/20/09

14 year Patent Term Cap Date: 9/20/09

EXHIBIT 9

U.S. PATENT 4,335,139

United States Patent [19]

Watts et al.

[11] 4,335,139

[45] Jun. 15, 1982

[54] PHARMACEUTICAL FORMULATIONS
CONTAINING PROSTACYCLIN
COMPOUNDS

[75] Inventors: Ian S. Watts, Sidcup; Peter H.
Marsden, Dartford, both of England

[73] Assignee: Burroughs Wellcome Co., Research
Triangle Park, N.C.

[21] Appl. No.: 182,054

[22] Filed: Aug. 28, 1980

Related U.S. Application Data

[63] Continuation of Ser. No. 39,645, May 16, 1979, abandoned.

[30] Foreign Application Priority Data

May 17, 1978 [GB] United Kingdom 20175/78

[51] Int. Cl.³ A61K 31/34

[52] U.S. Cl. 424/285

[58] Field of Search 424/305, 317, 285

[56] References Cited

U.S. PATENT DOCUMENTS

4,058,623 11/1977 Rolf-Rudiger

FOREIGN PATENT DOCUMENTS

2654149 6/1977 Fed. Rep. of Germany .

2720999 11/1977 Fed. Rep. of Germany .

2351112 4/1977 France .

1489780 10/1977 United Kingdom .

1503447 3/1978 United Kingdom .

1504070 3/1978 United Kingdom .

1504437 3/1978 United Kingdom .

OTHER PUBLICATIONS

Hayashi et al.—Chem. Abst., vol. 90 (1979), pp. 127, 526.

Shirley—Organic Chemistry (1964, Holt), pp. 535-536.

Finar—Organic Chemistry—3rd Edit. (Longmans, 1959), pp. 305-307.

Primary Examiner—Sam Rosen

Attorney, Agent, or Firm—Donald Brown

[57] ABSTRACT

Stabilized pharmaceutical formulations of prostacyclin or certain analogues thereof comprising an amino acid buffer, optionally containing a base, and the preparation of such formulations.

34 Claims, No Drawings

PHARMACEUTICAL FORMULATIONS CONTAINING PROSTACYCLIN COMPOUNDS

This is a continuation of application Ser. No. 039,645, filed May 16, 1979, now abandoned.

The present invention relates to pharmaceutical compositions containing prostacyclin (PGI₂, PGX), 15-methylprostacyclin, 16,16-dimethylprostacyclin, or their pharmaceutically acceptable salts.

Prostacyclin and its salts are important in human medicine and veterinary practice as they have a powerful anti-aggregating action on blood platelets and also accelerate wound healing and prevent, or have a therapeutic effect on, stomach ulcers.

The anti-aggregatory effect on blood platelets is useful, for example, in preventing or mitigating the formation of thrombi or emboli during extracorporeal circulation of blood, e.g. in renal dialysis and cardio-pulmonary by-pass.

However, a difficulty experienced with prostacyclin and its salts is that prostacyclin and its salts are unstable, especially in aqueous solution, and are quickly transformed into 6-oxo-PGF_{1α} and its salts, which are almost pharmacologically inactive and have very few, if any, of the beneficial activities of prostacyclin and its salts.

Prostacyclin and its salts are known to be more stable in alkaline media than in acidic or neutral media. Although some improvement in stability has been obtained by using tris buffer in alkaline solution, the prostacyclin has still been rapidly decomposed. This has meant that the prostacyclin has had to be made up very shortly before use by, say, intravenous infusion, and the pharmacological activity of the solution has changed considerably while it has been waiting to be used. This has made it difficult for the infusion to be controlled as carefully as desirable.

We have now surprisingly found that a marked improvement in stability can be obtained by having the prostacyclin, 15-methylprostacyclin, 16,16-dimethylprostacyclin, or a salt thereof, preferably the sodium salt, (hereinafter referred to as the "active compound"), in association with a pharmaceutically acceptable buffer having a pH value of at least 9 and based on an amino acid as the principal buffering acid in the buffer. The active compound and the buffer may be in association in the solid state and in solution in a solvent, usually water. When the active compound and buffer are in solution, the pH measured is that of the solution containing said active compound and buffer, and said formulation or buffer preferably has a pH of at least 9.

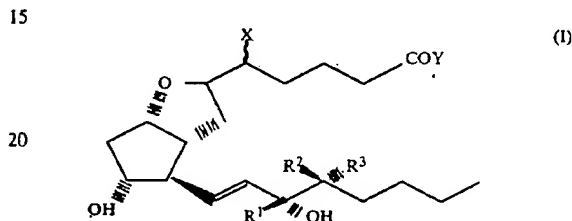
If the active compound and the buffer are in association in the solid state, by pH value of the buffer or the formulation we mean the pH measured in any one of the following ways. If the solid is a frozen solution, then the pH measured is that of the solution resulting from thawing the frozen solution. For other solid states, the pH measured is that of the solution resulting from dissolving the associated active compound and buffer in Water for Injections having a pH value of 7. For the particular case of a freeze dried residue of such a solution, the pH measured is that of the solution resulting from dissolving a sample of the residue in the minimum volume of Water for Injections having a pH value of 7 required to produce a clear solution.

In the solid state, the freeze drying of such a solution results in improved stability of the active compound. Freeze drying may be effected in the conventional man-

ner in, for example, an ampoule or vial. The solution may also be frozen and stored at, say, -20° C. for use as a frozen injection or for diluting on thawing.

Such a solution and all solutions hereinafter referred to are, for medicinal purposes, to be understood to be sterile solutions.

Prostacyclin and analogues thereof, in particular 15-methylprostacyclin and 16,16-dimethylprostacyclin, and a salt of one of these may be prepared by methods described in our co-pending UK Patent Application No. 19384/76 for the preparation of compounds of analogous structure. These include dehydrohalogenation of a compound of formula (I):



wherein X is bromo or iodo; Y is OH, NHR⁴ or OR, R being alkyl of 1 to 4 carbon atoms or a pharmaceutically acceptable cation such as sodium, R⁴ being alkyl of 1 to 4 carbon atoms; R¹, R² and R³ are independently selected from hydrogen and alkyl of 1 to 4 carbon atoms particularly methyl, with a base; for example, when R¹ is hydrogen R² and R³ are both methyl and vice-versa; and converting, if necessary, the resulting compound in which Y is NHR⁴ or OR, R and R⁴ being alkyl of 1 to 4 carbon atoms, into the desired active compound.

The amino-acid for use in the buffer is preferably sulphur-free and the most preferred amino-acid is glycine, especially as it is readily available and, if prepared synthetically, does not need to be optically resolved; but other amino-acids such as valine, alanine and arginine may also be used.

The total concentration of amino-acid (i.e. including its salts) in the buffer is preferably as low as is consistent with obtaining a stable buffer, e.g. in the range of from 0.02 M to 0.03 M, preferably about 0.025 M, as the presence of too much amino-acid tends to reduce the stability of the active compound by increasing the ionic strength of a solution of the associated active compound and buffer. The amino-acid must be sufficiently soluble to provide the necessary buffering capacity.

If sodium chloride is present in a solution of the buffer, as is preferred, the amount added should not be such that the solution is pharmaceutically unacceptable. The molar concentration of sodium chloride is preferably about the same as that of the amino-acid. Too much sodium chloride would raise the ionic strength of a solution of the associated active compound and buffer undesirably and adversely affect the stability of the active compound. Other salts, e.g. potassium chloride, may also be present if they, or their amounts, are pharmaceutically acceptable.

The pH of the buffer is preferably 10.2 to 11.6, especially about 10.5, when measured by the appropriate method as hereinabove described.

As an example of preparing a buffer solution according to the invention containing prostacyclin, a solution of glycine in water and also containing some sodium chloride was prepared. To this solution was added so-

dium hydroxide as base to raise the pH to the desired level and then the prostacyclin was added.

Although sodium hydroxide was used as base in the above example, any base may be used that is strong enough to give a buffer solution of the desired pH. Naturally the base should be one that gives rise to a pharmaceutically acceptable solution, i.e. one that is not deleterious to the recipient. The amount of amino-acid, for example, glycine and base, for example, sodium hydroxide, used should be as little as is necessary to stabilize the active compound for the period of time required. Use of excess of either or both amino-acid or base results in retention of water in a freeze dried product which brings about deterioration of the active compound. However, the pH of the solution is an important factor in assessing pharmaceutical acceptability. If the buffer solution is to be introduced into a machine for example in renal dialysis, then the pH can be up to 12 or even more, but if the buffer solution is to be administered in a large volume into a vein, for example in cardio-pulmonary by-pass, then the pH on entering the vein should preferably be in a range of from 8.4 to 9, and for this the pH of the buffer solution can be lowered shortly before use.

Other buffering agents may be present in the buffer solution but their amount should not substantially reduce the stability of the active compound in the solution. For example, some carbonate may be present, derived from the prostacyclin, e.g. prostacyclin sodium may contain up to 5% by weight of sodium carbonate.

As an active compound is very active pharmacologically, the amount of it needed is very small; for example only a few milligrams are needed for a one hour infusion into an average person of 70 kg body weight. The amount of active compound present in a given buffer solution before freeze drying depends on the projected use for the freeze dried material on reconstitution. The reconstitution may be with buffer solution free of active compound so that the ratio of active compound to buffering constituents may be much greater than in the solution used for administration.

If a solution containing only buffering agents, active compound and sodium chloride is freeze dried, the physical strength and appearance of the freeze dried plug obtained are not particularly satisfactory. It is accordingly preferred to include an excipient in the buffer solution before freeze drying the solution. The preferred excipient is mannitol. Preferably the concentration of excipient is from 25 to 50 mg/ml of buffer solution. For mannitol if less than 25 mg/ml is used there is insufficient improvement in strength and appearance. If more than 50 mg/ml is used there is little or no further improvement and the stability of the active compound may be adversely affected. The excipient provides, in the freeze dried plug, a supporting matrix and improves the physical strength and appearance of the plug. Not all excipients may be used; for example those producing excessive foaming of the reconstituted material in the freeze drying vial, e.g. polyvinylpyrrolidone, should be avoided. Also, others affecting the pH of the buffer, e.g. glycine itself, should be avoided.

The buffer solution may also contain, if desirable, other therapeutic material besides the active compound. A freeze dried product may also contain such other therapeutic material or these may be added to the reconstituted buffer solution. Such other therapeutic material may partly or completely replace the excipient.

The solvent used in preparing the buffer solution is preferably Water for Injections (European Pharmacopoeia) or other water suitable for use in infusions or injections. When reconstituting the freeze dried material for use, it may be redissolved in Water for Injections generally having a pH value in the range of from 5.5 to 7, preferably 7, or in amino-acid buffer solution having a pH of at least 9, preferably about 10.5, or perhaps some of the buffer solution may be added to a solution in Water for Injections. Reconstitution of the freeze dried material may also take place by dissolving it in an infusion base, such as physiological saline suitable for infusion. It is also possible to use glucose for this purpose.

The freeze drying of the buffer solution may be carried out in any conventional manner, with the water content of the plug being lowered as far as convenient to improve further the stability of the active compound.

The amount of active compound required for therapeutic effect varies with the route of administration. In general, a suitable dose for a mammal will lie in the range of from 0.01 to 200 mg. per kilogram body weight, conveniently of from 0.01 to 10 mg/kg, preferably of from 0.1 to 1.0 mg/kg, and especially of from 0.2 to 0.5 mg/kg.

The amount of active compound present in an ampoule for administration by infusion will lie in the range of from 0.1 to 1.5 mg/kg, preferably of from 0.5-1.0 mg/kg. When used in man, the active compound may be several times more potent than in other mammals, and accordingly it may be desirable to use doses which appear at the lower ends of the dose ranges given hereinabove.

For human and veterinary use, it may be convenient to provide a collection of at least two vessels, for example as a multicomponent pack; one of which is a vial or ampoule containing a freeze dried (lyophilised) plug of the buffered active compound as described hereinabove, another vessel of which is a vial or ampoule containing a further amount of the buffer in aqueous solution or freeze dried which does not contain the active compound. The freeze dried product may then be reconstituted with the aqueous buffer, or where the contents of the second vessel are freeze dried, with a suitable aqueous diluent from a third vessel. The reconstituted material may then be diluted further, if required, to provide the desired dosage immediately prior to administration. Thus a freeze dried preparation of, for example 0.5 mg active compound, at pH 11.5 may be diluted, with 50 or 500 ml of aqueous dextrose or saline solution having a pH such that the resultant solution has a pH of 10.0 to 10.5.

Accordingly the present invention provides at least the following:

(a) A pharmaceutically acceptable buffer solution which comprises prostacyclin, 15-methylprostacyclin, 16,16-dimethylprostacyclin or a salt of any one of these, and, as principal buffering acid, an amino-acid, the solution having a pH of at least 9;

(b) A method of preparing a buffer solution according to (a), which comprises bringing the ingredients into solution in a suitable solvent;

(c) A freeze dried material obtained by freeze drying a buffer solution according to (a);

(d) A frozen injection obtained by freezing a buffer solution according to (a);

(e) A solution suitable for injection or infusion obtained by dissolving a freeze dried material according to

(c) in a suitable solvent, or thawing a frozen injection according to (d);

(f) A method for administering prostacyclin, 15-methylprostacyclin, 16,16-dimethylprostacyclin, or a salt of any one of these which comprises administering a solution according to (a), (d) or (c).

(g) A pharmaceutical formulation comprising an active compound selected from prostacyclin, 15-methylprostacyclin, 16,16-dimethylprostacyclin and a salt of any one of these in association with a pharmaceutically acceptable buffer having a pH of at least 9 and based on an amino acid as principal buffering acid in the buffer.

(h) A pharmaceutical formulation comprising an active compound selected from prostacyclin, 15-methylprostacyclin, 16,16-dimethylprostacyclin and a salt of any one of these in association with a pharmaceutically acceptable buffer based on an amino acid as the principal buffering agent.

The present invention is illustrated by the following Examples:

EXAMPLE 1

Sterile solutions containing prostacyclin (0.2 and 0.4 mg/ml) and mannitol (50 mg/ml) were prepared in the following buffers: glycine, arginine, valine, alanine, carbonate, and tris. The concentrations of the buffering components were:

Amino acid buffer: 0.025 M amino acid, 0.025 M sodium chloride and sodium hydroxide q.s. pH 10.5.

Carbonate buffer: 448 mg/l sodium bicarbonate and 1614 mg/l sodium carbonate.

Tris buffer: 6054 mg/l of tris base and 2.5 ml of 0.1 N sodium hydroxide per liter.

5 ml portions of each of these solutions were then freeze dried in vials by freezing at -40°C . and under vacuum, carrying out first stage drying at 0°C . and second stage drying at 20°C . Sealing of the vials was conducted in a nitrogen atmosphere. The freeze dried products were then subjected to an accelerated storage test at the temperature shown in Table I. The products obtained after the times indicated in Table I were analysed for their prostacyclin content by high performance liquid chromatography (HPLC). The HPLC was carried out on freeze dried preparations reconstituted in 10 ml of 0.025% tetramethylammonium hydroxide solution.

The column used was a 25 cm \times 4.2 mm I.D. stainless steel one filled with laboratory prepared Partisil ODS packing material made as given below. The column was packed using the method of Webber and McKerral, J. Chromatog., 122, 243, (1976), employing carbon tetrachloride as the slurry medium.

The column packing material was prepared as follows; 10 μm Partisil (10 g) was dried at 80°C . in vacuo for two and half hours in a 250 ml round bottomed flask. Octadecyltrichlorosilane (10 ml) and dry toluene (100 ml) were added and the solution was refluxed for three hours with paddle stirring using a reflux condenser fitted with a calcium chloride guard tube. The mixture was allowed to cool and then filtered through a 0.5 μm millipore filter. The silica in the filter was washed with 250 ml methanol, slurring the solid continuously, then with 250 ml hot acetone and dried at 80°C . in vacuo for about two hours. The product (11 g) was treated with trimethylchlorosilane (10 ml) as above, refluxing for 45 minutes to give the final product.

The mobile phase used was water (1200 ml) in which was dissolved 5 g boric acid and 7.6 g di-sodium tetra-

borate and then methanol (800 ml) was added. The column temperature used was ambient i.e. about 25°C . to 30°C ., and the mobile phase flow rate was 3.6 ml/min. and the pressure used was 20 MPa. Detection of the products was carried out using a Pye Unicam LC3 at 205 nanometers wavelength, 0.16 a.u.s. (absorbance units full scale) for 0 to 100 $\mu\text{g/ml}$ solutions.

The amount of prostacyclin present was determined by peak height measurement and comparison with a reference sample of known concentration.

The results obtained are set out in Table I.

TABLE I

Buffer	HPLC assay (% prostacyclin remaining)				
	66 hours				6 days (144 hours)
	70°C .	60°C .	50°C .	37°C .	26°C .
Glycine	0	0	3	90	96
Arginine					
Valine					
Alanine					
Carbonate	0	2	3.5	45	62
Tris	0	0	trace	trace	35

It can be clearly seen from Table I that at normal storage temperatures, i.e. about 37°C . or below, the amino-acid buffers, especially glycine, are superior to the other two buffers tested. Although the carbonate buffer is inferior to the amino-acid buffer it is clear that some carbonate could be tolerated in the amino-acid buffer.

EXAMPLE 2

Freeze dried Injection of prostacyclin (1mg)	
Prostacyclin	1.000 mg
Mannitol	50.000 mg
NaCl (0.025M)	2.932 mg
Glycine (0.025M)	3.760 mg
NaOH	q.s. to pH 10.5

Using the general procedure give in Example 1, a 1 mg prostacyclin injection comprising the above ingredients was freeze dried to give a residue which may contain up to 5% w/w of water.

EXAMPLE 3

A stirred solution of $\text{PGF}_{2\alpha}$ methyl ester (50 mg) in ether (1 ml) was treated with sodium bicarbonate (115.0 mg; 10 molecular equivalents) and water (1 ml) and then dropwise during 2 hours with aqueous potassium triiodide (0.7 molar; 0.261 ml). After stirring overnight, the reaction mixture was shaken with ether and aqueous sodium thiosulphate; the ethereal phase was separated, washed with water, dried with magnesium sulphate, and evaporated to leave a yellow gum of 5 ξ -iodo-9-deoxy-6 ξ ,9 α -epoxyprostaglandin $\text{F}_{1\alpha}$ methyl ester.

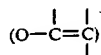
A solution of 5 ξ -iodo-9-deoxy-6 ξ ,9 α -epoxyprostaglandin $\text{F}_{1\alpha}$ methyl ester (100 mg) in methanolic sodium methoxide prepared from sodium (46 mg) and dry methanol (0.70 ml) was set aside under dry nitrogen for 5 hours, then freed from solvent in high vacuum. The residual amorphous solid was washed with benzene, set aside in the air overnight, and stirred with N aqueous sodium hydroxide (0.5 ml) to give a suspension of colourless fine needles. The crystals were collected, washed with a few drops of N aqueous sodium hydroxide, and dried in the air to give the sodium salt of 9-deoxy-6,9 α -epoxy- Δ^5 -prostaglandin $\text{F}_{1\alpha}$. The inhibition

of arachidonic acid-induced aggregation of human platelets at a concentration of 0.2 ng/ml by this salt and its instability in water at acid pH, together with further evidence, is compatible with assignation of the configuration (5Z)-5,6-didehydro-9-deoxy-6,9 α -epoxyprostaglandin F_{1 α} .

The high-resolution ¹³C n.m.r. spectrum of a solution of the crystals in dimethyl sulphoxide-d₆ showed the expected 20 resonances whose chemical shifts were entirely consistent with the chemical structure established for Prostacyclin. No impurity peaks were detected.

EXAMPLE 4

5 ξ -Iodo-9-deoxy-6 ξ ,9 ξ -epoxyprostaglandin F_{1 α} methyl ester (500 ml) was stirred with methanolic NaOMe prepared from Na (0.23 g, 10 equivs.) and MeOH (3.5 ml) under N₂ at room temperature overnight; 1 N aq. NaOH (2.5 ml) was added to the yellow reaction solution to bring about hydrolysis of the ester moiety and, after 2 hours, the methanol was evaporated in vacuo at room temperature. The residual aqueous solution gave rise spontaneously to a mass of colourless fine needles of the desired sodium salt which was cooled (0°), collected, washed sparingly with 1 N aq. NaOH, air-dried, and stored in a stoppered tube; this salt (383 mg) had ν max (KBr disc) 1692 cm⁻¹.

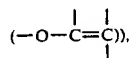


and twenty ¹³C resonances only were observed at 182.7 (C-1), 158.2 (C-6), 140.0 and 134.3 (C-13,14), 100.7 (C-5), 87.5 (C-15), 80.6 and 75.5 (C-9,11), 58.0 (C-12), 49.0, 45.8, 42.4, 41.9, 37.5, 35.8 (C-18), 31.6, 29.9, 29.3, 26.7 (C-19), 18.4 (C-20) ppm from TMS in DMSO-d₆. The product was sodium (5Z)-5,6-didehydro-9-deoxy-6,9 α -epoxyprostaglandin F_{1 α} (syn. sodium prostacyclin).

EXAMPLE 5

5 ξ -Iodo-9-deoxy-6 ξ ,9 α -epoxyprostaglandin F_{1 α} methyl ester was treated with 1,5-diazabicyclo-5-nonene (DBN) at room temperature in the absence of a solvent for a few hours.

The DBN and hydrogen iodide were conveniently removed by adsorption on to a column of SiO₂, prepared from a suspension of SiO₂ in EtOAc/Et₃N 50:1, and the vinyl ether was eluted with the same solvent system. I.R. spectroscopy (thin film, ν max 1738 (CO₂Me) and 1696 cm⁻¹



¹H n.m.r. in C₆D₆-Et₃N, 19:1 (84.22, triplet of triplets¹², J_{6,9} and 1.0 Hz (C-5 vinyl proton)), and ¹³C n.m.r. in C₆D₆-Et₃N, 19:1 (distinctive features were resonances at 159.8 (C-1), 155.8 (C-1), 155.8 (C-6), 137.2 and 130.6 (C-13,14), 95.3 (C-5), 84.1 (C-15) 77.3 and 72.2 (C-9,11) and 51.1 (Me ester) ppm from TMS).

The vinyl ether (5Z)-5,6-didehydro-9-deoxy-6,9 α -epoxyprostaglandin methyl ester, was hydrolysed with aqueous sodium hydroxide to give synthetic sodium prostacyclin.

EXAMPLE 6

(5R, 6R)-5-Iodo-PGI₁ methyl ester (13.375 g, containing ca.2% 5S, 6S isomer) was taken up in methanolic sodium methoxide [from Na (6.23 g) and MeOH (94 ml)] at room temperature under N₂ and set aside at room temperature overnight. The resulting yellow solution was treated with 1 N aqueous NaOH (70 ml), filtered from sediment, set aside at room temperature for 2 hours, and freed from MeOH on a Buchi evaporator in vacuo at room temperature. The residual syrup was treated with H₂O (25 ml) and with more 1 N aqueous NaOH (80 ml), crystallisation taking place spontaneously to give a mass of felted crystals. After cooling to 0°, the solid was collected, washed with ice cold 1 N aqueous NaOH (ca. 40 ml) until the washings were colourless, and dried in the air (2 days) to constant weight, affording 10.15 g, m.p. 164°-166° (following drying at 100°) of colourless prostacyclin sodium salt.

EXAMPLE 7

Sterile Diluent for Injection of Prostacyclin	
Glycine (0.025M)	94.0mg
NaCl (0.025M)	73.3mg
NaOH	q.s. to pH 10.5
Water for Injections up to	50ml.

Using the general procedure described in Example 1, the above ingredients were used in a solution for use as a diluent.

We claim:

1. A pharmaceutical formulation comprising an active compound selected from prostacyclin, 15-methylprostacyclin, 16, 16-dimethylprostacyclin or a pharmaceutically acceptable salt of any one of these in association with a pharmaceutically acceptable buffer having a pH of at least 9 and based on a pharmaceutically acceptable amino acid as a buffering acid in the buffer and, optionally, a further pharmaceutically acceptable carrier.

2. A formulation according to claim 1, wherein the further pharmaceutically acceptable carrier is or includes a solvent which dissolves the active compound and the buffer, to form a solution.

3. A formulation according to claim 1, wherein the further pharmaceutically acceptable carrier is or includes water.

4. A formulation according to claim 1, wherein a solution containing the active compound and the buffer is suitable for injection or infusion.

5. A formulation according to claim 1, wherein a solution containing the active compound and the buffer is freeze dried or frozen.

6. A formulation according to claim 1, wherein the amino-acid is sulphur-free.

7. A formulation according to claim 1, wherein the amino-acid is selected from the group consisting of glycine, alanine, arginine and valine.

8. A formulation according to claim 1, wherein the total concentration of the amino-acid including its salts, is in the range of from 0.02 to 0.03 M.

9. A formulation according to claim 1, wherein the total concentration of the amino-acid, including its salts, is about 0.025 M.

10. A formulation according to claim 1, wherein the further pharmaceutically acceptable carrier comprises sodium chloride.

11. A formulation according to claim 1, wherein the pH of the buffer is in the range of from 10.2 to 10.8.

12. A formulation according to claim 1, wherein the further pharmaceutically acceptable carrier comprises an excipient.

13. A formulation according to claim 1, wherein the further pharmaceutically acceptable carrier comprises mannitol.

14. A formulation according to claim 1, wherein the active compound is prostacyclin or a salt thereof.

15. A formulation according to claim 1, wherein the salt is a sodium salt.

16. A formulation according to claim 1, wherein the active compound is prostacyclin sodium salt.

17. A formulation according to claim 1, comprising prostacyclin sodium salt in association with glycine and optionally, a further pharmaceutically acceptable carrier.

18. A method of preparing a pharmaceutical formulation according to claim 1, which comprises bringing an active compound selected from prostacyclin, 15-methylprostacyclin, 16,16-dimethylprostacyclin, and a salt of any one of these into association with a pharmaceutically acceptable buffer having a pH of at least 9 and based on an amino acid as principal buffering acid in the buffer and, optionally, a further pharmaceutically acceptable carrier.

19. A formulation according to claim 1, when prepared by a method according to claim 18.

20. A method of inhibiting the aggregation of platelets, which comprises the bringing of said platelets into association with an amount of a formulation according to claim 1 containing an effective platelet aggregation inhibitory amount of the active compound.

21. A method of inducing vasodilation in a mammal, which comprises the administration to said mammal of an amount of a formulation according to claim 1 containing a non-toxic, effective vasodilatory amount of the active compound.

22. A method for the treatment or prophylaxis of thrombosis in a mammal or mammalian tissue which comprises administration of an amount of a formulation according to claim 1 containing a non-toxic, effective antithrombotic amount of the active compound.

23. A method of lowering blood pressure in a mammal which comprises administration to said mammal of

an amount of a formulation according to claim 1 containing a non-toxic, effective hypotensive amount of the active compound.

24. A method for the treatment or prophylaxis of a gastric lesion in a mammal which comprises administration to said mammal of an amount of a formulation according to claim 1 containing a non-toxic, effective therapeutic or prophylactic amount of the active compound.

25. A method of stabilizing an active compound selected from the group consisting of prostacyclin, 15-methylprostacyclin, 16,16-dimethylprostacyclin and a pharmaceutically acceptable salt of any one of these against hydrolysis of the enol ether moiety, which method comprises bringing said compound selected from the group consisting of prostacyclin, 15-methylprostacyclin, 16,16-dimethylprostacyclin, and a pharmaceutically acceptable salt thereof into association with a pharmaceutically acceptable buffer having a pH of at least 9 and based on an amino acid as a buffering acid in the buffer.

26. A pharmaceutical formulation comprising an active compound selected from prostacyclin, 15-methylprostacyclin, 16,16-dimethylprostacyclin and a salt of any one of these in association with a pharmaceutically acceptable buffer based on an amino acid as principal buffering agent and buffered to a pH of at least 9.

27. A formulation according to claim 26, wherein the formulation further includes a base.

28. A pharmaceutical composition comprising prostacyclin or a pharmaceutically acceptable salt thereof in association with a pharmaceutically acceptable buffer having a pH of at least 9 and based on an amino acid as the principal buffering acid in the buffer.

29. The composition of claim 28 in which the amino acid is arginine.

30. The composition of claim 28 in which the amino acid is valine.

31. The composition of claim 28 in which the amino acid is alanine.

32. The composition of claim 28 in a form for injection, said composition including water.

33. A pharmaceutical composition comprising the sodium salt of prostacyclin in association with a pharmaceutically acceptable buffer having a pH of at least 9 and based on glycine as the acid in the buffer.

34. The composition of claim 33 in a form for injection, said composition including water.

* * * * *

EXHIBIT 10

U.S. PATENT 4,539,333

United States Patent [19]

Moncada

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[45] Date of Patent: Sep. 3, 1985

[54] PROSTACYCLIN, METHODS OF USING
AND METHOD OF MAKING

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C07D 307/935

[52] U.S. Cl. 514/469; 435/63;
549/465

[58] Field of Search 260/346.22; 424/285;
435/63; 549/465

[56] References Cited

U.S. PATENT DOCUMENTS

4,158,667	6/1979	Axen	562/503
4,338,325	7/1982	Johnson et al.	549/465

OTHER PUBLICATIONS

Pace-Asciak et al., *Biochemistry*, vol. 10, No. 20,
(1971), pp. 3657-3664.

Corey et al., *J.A.C.S.*, 99(6), Mar. 16, 1977, pp.
2006-2008.

Johnson et al., *Prostaglandins*, vol. 12(6), Dec. 1976, pp.
915-928.

Pace-Asciak et al (II), *Prostaglandins*, Sep. 1978, vol.
16, No. 3, pp. 397-410.

Shirley, *Organic Chemistry*, Holt, Rinehart and Win-
ston, (1946), p. 353.

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[57] ABSTRACT

Prostacyclin, its salts, biosynthesis and synthesis
thereof, pharmaceutical formulations containing them,
and their use in medicine.

34 Claims, No Drawings

PROSTACYCLIN, METHODS OF USING AND METHOD OF MAKING

This invention relates to the extraction, isolation and synthesis of a prostaglandin derivative, its use as a chemical intermediate, formulations containing it, and its use in medicine.

Prostaglandin endoperoxides (PGG₂ and PGH₂) are generated from arachidonic acid by a membrane-bound cyclo-oxygenase enzyme system, described by Hamberg and Samuelsson (Biochem. Biophys. Acta. 326, 448-461, 1974) and are subsequently transformed to PGF_{2α}, PGE₂, PGD₂, or Thromboxane A₂. Thromboxane A₂ shares with the prostaglandin endoperoxides the important biological properties of contracting strips of rabbit aorta and aggregating blood platelets.

The Applicants have now found that microsomes derived from a variety of mammalian tissues catalyze the enzymic transformation of the prostaglandin endoperoxides to a prostaglandin derivative (hereinafter referred to as Prostacyclin which does not contract strips of rabbit aorta, relaxes strips of rabbit coeliac mesenteric and coronary arteries, has a potent anti-aggregatory action on blood platelets, is a strong vasodilator in whole animals and has other properties described hereinafter.

Microsomes derived from rabbit or pig blood vessels such as veins and arteries, and rat stomach fundus produce about 80-90% conversion of the prostaglandin endoperoxides. Microsomes derived from rabbit lung tissue and rat pyloric tissue produce 25% conversion of the prostaglandin endoperoxides, whereas those derived from rat kidney, brain, spleen, liver, heart and seminal vesicle tissues produce 5% or less conversion of the prostaglandin endoperoxides.

Prostacyclin is unstable at room temperature in aqueous medium, having a half-life of approximately 10 mins., but its anti-aggregatory activity can be preserved for several days by dissolving the substance in aqueous alkali or in dry acetone and storing at -20° C. On average prostacyclin is 10-40 times more potent as an anti-aggregatory agent than PGE₁ and 5-20 times more potent than PGD₂, itself a potent inhibitor of platelet aggregation. Prostacyclin also interrupts and reverses the presence of platelet aggregation.

Prostacyclin may be prepared biosynthetically by incubating PGG₂ or PGH₂ with aortic microsomes in a suitable buffer solution such as Tris buffer, for approximately 2 minutes at a temperature in the region of 22° C. Conversion of the prostaglandin endoperoxides is approximately 85%.

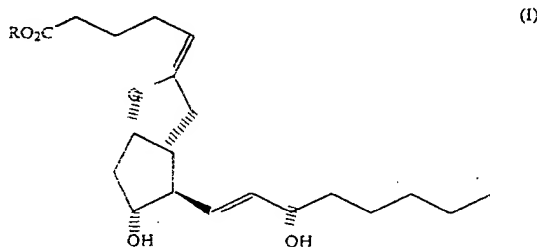
Extraction of Prostacyclin is achieved by the addition of cold (0° C.) dry diethyl ether to the incubation mixture. The addition of cold ether stops the enzyme reaction and Prostacyclin enters the ether phase which may be separated from the aqueous phase. Evaporation of the ether by standard techniques such as bubbling nitrogen through the solution results in the Prostacyclin being left as a residue which may subsequently be resuspended in an aqueous solution for further examination or dissolved in anhydrous acetone and stored at a temperature in the region of -20° C. for future use.

The aortic microsomes employed in the incubation mixture may be extracted from pig or rabbit aortas. Aortas may be frozen solid preferably by dropping them in liquid nitrogen, and then crushed to form a powder which is resuspended in a suitable buffer solu-

tion and subsequently homogenized. The homogenate may then undergo sequential centrifugation so as to isolate the microsomal fraction which may be resuspended in deionized water and lyophilized. The incubation mixture was shown to have an immediate anti-aggregatory effect by monitoring the aggregation of human blood platelets in a Born aggregometer.

Prostacyclin may be prepared using other tissues identified above in substantially a similar manner. Prostacyclin formed in such an incubation mixture appears to be different from the other products of PG endoperoxides so far described. Its biological properties on the isolated tissues, its instability and its potent anti-aggregatory activity show that Prostacyclin is not being PGE₂ or PGE_{2α}. The presence of prostaglandin D₂ isomerase in homogenates of several tissues has been described. As prostacyclin is unstable and is a more potent anti-aggregatory agent than PGD₂, it cannot be regarded as PGD₂. Furthermore, PGD₂ isomerase is present in the 100,000 g. supernatant, a fraction that did not produce Prostacyclin from PG endoperoxides. Moreover, PGD₂ isomerase needs glutathione as a cofactor and the incubations were carried out in the absence of cofactors. PGE₂, PGF_{2α} and PGD₂ were not substrates for aortic microsomes and therefore 15-keto PGs and other products of prostaglandin catabolism could not be considered as Prostacyclin. Prostacyclin is also unlikely to be a known 15-hydroperoxy PG, firstly because 15-hydroperoxy PGE₂ has a contractile activity on rabbit aortic strip and secondly the product(s) of the spontaneous decay of Prostacyclin then bio-assayed did not behave like PGE₂, PGF_{2α}, or PGD₂. As Prostacyclin has an anti-aggregatory activity it cannot be identical to Thromboxane A₂ or B₂ as they are pro-aggregatory substances.

Further studies have shown the Prostacyclin has the chemical structure shown in formula (I) (R is hydrogen) (see Johnson et al., Prostaglandins, 12/6, 915-928, 1976).

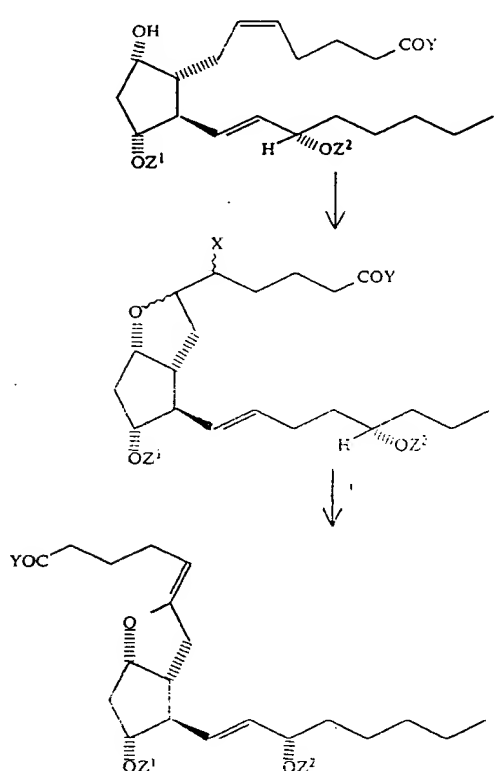


By the present invention we provide the compounds of formula (I) wherein R is hydrogen or a pharmacologically acceptable cation (hereinafter referred to as Prostacyclin and salts thereof). Salts of Prostacyclin include alkali metal salts, alkaline earth metal salts and salts of organic bases.

Salts of Prostacyclin may be synthesised from a compound of formula (II) wherein either or both of Z¹ and Z² is hydrogen or a blocking group such as acyl, or trialkylsilyl (for example trimethylsilyl). Oxidative attack by iodine or potassium triiodide in the presence of a metal bicarbonate at the 5,6-double bond of a compound of formula (II) with simultaneous or subsequent cyclisation involving the 9-hydroxy group produces a compound of formula (III). Upon treatment with a suitable base such as an organic base or a metal alkoxide,

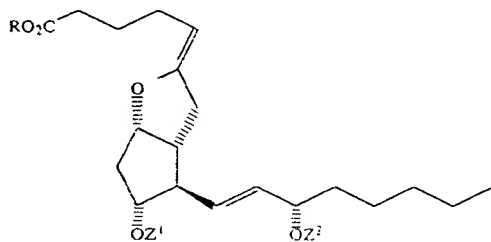
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a compound of formula (III) may then be dehydrohalogenated, resulting in the introduction of a 5,6-double bond. This reaction sequence is illustrated in the following reaction scheme:



wherein Y is OH, NHR¹ or OR¹, R¹ being alkyl of 1 to 4 carbon atoms or a cation; X is iodo or bromo; and Z¹ and Z² are as defined above.

Included in compounds of formula (IV) are compounds of formula



wherein R is hydrogen or a pharmacologically acceptable cation and each of Z¹ and Z² is hydrogen or a blocking group.

When Z and/or Z² in formulae (II), (III) and (IV) are blocking groups, the resulting blocked derivatives of the compounds of formula (I) may be converted to the corresponding compounds of formula (I) by methods known in the art, for example base hydrolysis.

Prostacyclin salts may be prepared by treating an ester thereof (formula (I): R is alkyl) for example the methyl ester, with a strong base such as sodium hydroxide in a suitable solvent and lyophilising the resulting reaction mixture. Prostacyclin itself may be conveniently

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prepared by base hydrolysis of its corresponding esters or amides in the presence of an equivalent amount of a caustic alkali in an aqueous alcohol or an aqueous tetrahydrofuran medium, and extracted into an organic solvent at low temperature.

Prostacyclin and its salts are useful as intermediates in the synthesis of prostaglandin analogues, and exhibit a potent anti-aggregatory action on blood plates, and therefore have a particular utility in the treatment and/or prophylaxis of mammals as anti-thrombotic agents.

They are also useful in mammals, including man, to reduce and control excessive gastric secretion, thereby reducing or avoiding gastrointestinal ulcer formation, and accelerating the healing of such ulcers and lesions already present in the gastrointestinal tract.

Prostacyclin and its salts further exhibit vasodilatory action on blood vessels and therefore have a particular utility as anti-hypertensives for the treatment of high blood pressure in mammals, including man. Platelets can be assimilated into the vascular endothelium or even incorporated into endothelial cells. Biochemical co-operation between platelets and vascular endothelium in the generation of Prostacyclin contributes to the repair of vascular endothelium, and Prostacyclin and its salts have a further utility in the promotion of wound healing in mammals, including man.

Prostacyclin and its salts may be used whenever it is desired to inhibit platelet aggregation, to reduce the adhesive character of platelets, and to treat or prevent the formation of thrombi in mammals, including man. For example, they may be used in the treatment and prevention of myocardial infarcts, in the treatment of peripheral vascular disease to treat and prevent post-operative thrombosis, to promote patency of vascular grafts following surgery, and to treat complications of arteriosclerosis and conditions such as atherosclerosis, blood clotting defects due to lipemia, and other clinical conditions in which the underlying etiology is associated with lipid imbalance or hyperlipidemia.

They may also be used as additives to blood, blood products, blood substitutes, and other fluids which are used in artificial extra-corporeal circulation and perfusion of isolated body portions, e.g., limbs and organs, whether attached to the original body, detached and being preserved or prepared for transplant, or attached to a new body. During these circulations and perfusions, aggregated platelets tend to block the blood vessels and portions of the circulation apparatus. This blocking is avoided by the presence of Prostacyclin. For this purpose, Prostacyclin or its salts may be added generally or in single or multiple portions to the circulating blood, to the blood of the donor animal, to the perfused body portion, attached or detached to the recipient, or to two or all of those at a total steady state dose of 0.001 to 10 mg., per liter of circulating fluid. It is especially useful to use Prostacyclin in laboratory animals e.g. cats, dogs, rabbits, monkeys and rats, for these purposes in order to develop new methods and techniques for organ and limb transplants.

The amount of prostacyclin or a salt thereof required (hereinafter referred to as the active ingredient) for therapeutic effect will vary with the route of administration. In general a suitable dose for a mammal will lie in the range of 0.01 to 200 mg. per kilogram body-weight, conveniently 0.01 to 10 mg per kilogram.

While it is possible for the active ingredient to be administered as the raw material it is preferable to pres-

ent it as a pharmaceutical formulation. Such formulations are preferably non-aqueous and non-hydroxylic in nature, but alkaline aqueous solutions may be used. Unit doses of a formulation contain between 0.5 mg. and 1.5 g of the active ingredient.

Such formulations, both for veterinary and for human medical use, of the present invention comprise the active ingredient, as above defined, together with one or more acceptable carriers therefor and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The formulations include those suitable for parenteral (including subcutaneous, intramuscular and intravenous) administration which must of course be sterile.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired formulation.

According to the present invention there are therefore provided:

- (a) the compounds of formula (I) and derivatives thereof blocked in the 11- and 15-positions;
- (b) the preparation of Prostacyclin comprising:
 - (i) incubation of microsomes of fresh mammalian tissue with a prostaglandin endoperoxide, and the extraction of prostacyclin from the incubation mixture into an organic solvent; or
 - (ii) its synthesis as hereinbefore described;
- (c) Prostacyclin when obtained by the process described in paragraph (b);
- (d) a pharmaceutical formulation containing Prostacyclin;
- (e) the preparation of such pharmaceutical formulations;
- (f) method for the treatment of prophylaxis of thrombosis in a mammal or mammalian tissues, including man, comprising the administration of a non-toxic, prophylactic anti-thrombotic amount of a compound of formula (I);
- (g) method for inducing vasodilation in a mammal, including man, comprising the administration of a non-toxic, vasodilatory amount of a compound of formula (I);
- (h) method for the prophylaxis or treatment of gastric lesions in a mammal, including man comprising the administration of a non-toxic, prophylactic or therapeutic amount of a compound of formula (I);
- (i) (5Z)-5,6-didehydro-9-deoxy-6,9 α -epoxyprostaglandin F_{1 α} and its salts
- (j) method for the promotion of wound healing in a mammal, including man, comprising the administration of a non-toxic wound-treatment amount of a compound of formula (I).

The following examples are provided by way of an illustration of the present invention and should in no way be construed as constituting a limitation thereof.

EXAMPLE 1

Preparation of Prostacyclin

Pig aortas were stripped of adventitia, snap frozen in liquid nitrogen, crushed into a fine powder, resuspended in 0.05 M Tris buffer (pH 7.5) (1:4, w:v) and homogenised at highspeed in a Polytron (KIMATIC, LUCERNE, SWITZERLAND) homogenizer. The homogenate was centrifuged at 1000 \times g for 15 minutes and the resulting supernatant centrifuged again at 10,000 \times g for 5 minutes. The 10,000 \times g pellet was discarded, while the pellet obtained after centrifugation of the supernatant at 100,000 g for 60 minutes was resuspended in deionized water and lyophilized. An average yield of 150 mg. of aortic microsomal powder (51% protein) per 100 g. of aortic tissue was obtained.

Aortic microsomal powder (5 mg) was incubated with 1 μ g of prostaglandin endoperoxide (PGG₂ or PGH₂) in 0.05 M Tris buffer (pH 7.5) (1 ml.) for 2 minutes at 22° C. Enzyme activity was estimated by direct bioassay of the incubation mixture. After incubation of 2 minutes at 22° C. all prostaglandin endoperoxide activity was lost indicated by a lack of contraction of rabbit aorta strips when assayed by the cascade superfusion technique of Vane (Br. J. Pharmacology, 23, 369-373, 1964 indicating 100% conversion of PGG₂ or PGH₂).

The Prostacyclin was extracted by the addition of cold dry diethyl ether (1 ml) to the incubation mixture which also stopped the enzymic reaction. The Prostacyclin entered the ether phase, which was subsequently separated from the aqueous phase. The ether was evaporated by bubbling nitrogen through it leaving the Prostacyclin which was either dissolved in ice-cold 0.05 M Tris buffer (0.5-1 ml.) and immediately used for platelet aggregation studies or was dissolved in anhydrous acetone (1 ml.) and stored at -20° C. for future use.

The anti-aggregatory activity of the extracted Prostacyclin disappeared on boiling (15 seconds) or on standing at 22° C. for 20 minutes. The anti-aggregatory activity of Prostacyclin could be preserved for several days by dissolving the substance in dry acetone and storing at -20° C.

By using rabbit aortas in a similar experiment the same results were obtained.

EXAMPLE 2

Aggregation of platelets in 1 ml. of fresh human platelet rich plasma (PRP) was monitored in a Born aggregometer. An immediate anti-aggregatory effect of the fresh reaction mixture of Example 1 of aortic microsomes with PGH₂ or PGG₂ was observed. The lowest anti-aggregatory concentration was obtained with samples containing 0.5-5 ng. Prostacyclin/ml.

This activity disappeared after leaving the incubation mixture for 20 minutes at 22° C. or by boiling for 15 seconds. Aortic microsomes alone (50 μ g/ml) could induce aggregation in some PRP. The products of spontaneous degradation of PGG₂ (100 ng/ml) had no anti-aggregatory activity.

Diethyl ether extracts of Prostacyclin also inhibited platelet aggregation induced by arachidonic acid and PGG₂. The effective anti-aggregatory concentration of Prostacyclin after storage in ether was from 1 to 10 ng/ml.

EXAMPLE 3

Prostacyclin was injected intravenously and intra-aortically into normotensive anaesthetised rats and the blood pressure and heart rate recorded. Results showed Prostacyclin to be a powerful vasodepressor having 5 times the potency of PGE₂.

EXAMPLE 4

Prostacyclin was found to relax spirally cut strips of 10 coronary artery from the ox. The effect is dose dependent and relaxation can be seen with doses as low as 20 nanograms per 5 ml bath. In isolated hearts from rabbits perfused at constant flow rate by the Langendorf technique, Prostacyclin produced coronary vasodilation.

Prostacyclin relaxed strips of coeliac and mesenteric arteries, but was less potent than PGE₂.

EXAMPLE 5

Prostacyclin was shown to inhibit the formation of 20 gastric lesions induced in rats by indomethacin at doses from 62.5 to 250 µg/kg. upon subcutaneous injection

EXAMPLE 6

A stirred solution of PGF_{2α} methyl ester (50 mg) in 25 ether (1 ml) was treated with sodium bicarbonate (115.0 mg; 10 molecular equivalents) and water (1 ml) and then dropwise during 2 hours with aqueous potassium triiodide (0.7 molar; 0.261 ml). After stirring overnight, the reaction mixture was shaken with ether and aqueous 30 sodium thiosulphate; the ethereal phase was separated, washed with water, dried with magnesium sulphate, and evaporated to leave a yellow gum of 5ξ-iodo-9-deoxy-6ξ, 9α-epoxyprostaglandin F_{1α} methyl ester.

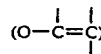
A solution of 5ξ-iodo-9-deoxy-6ξ, 9α-epoxyprosta- 35 glandin F_{1α} methyl ester (100 mg.) in methanolic sodium methoxide prepared from sodium (46 mg.) and dry methanol (0.70 ml.) was set aside under dry nitrogen for 5 hours, then freed from solvent in high vacuum. The residual amorphous solid was washed with benzene, set 40 aside in the air overnight, and stirred with N aqueous sodium hydroxide (0.5 ml.) to give a suspension of colourless fine needles. The crystals were collected, washed with a few drops of N aqueous sodium hydroxide, and dried in the air to give the sodium salt of 9-deoxy-6,9α-epoxy-Δ⁵-prostaglandin F_{1α}. The inhibition 45 of arachidonic acid-induced aggregation of human platelets at a concentration of 0.2 ng./ml. by this salt and its instability in water at acid pH, together with further evidence, is compatible with assignation of the configuration (5Z)-5,6-didehydro-9-deoxy-6,9α-epoxy- 50 prostaglandin F_{1α} sodium salt.

The high-resolution ¹³C n.m.r. spectrum of a solution of the crystals in dimethyl sulphoxide-d₆ showed the 55 expected 20 resonances whose chemical shifts were entirely consistent with the chemical structure established for Prostacyclin. No impurity peaks were detected.

EXAMPLE 7

5ξ-Iodo-9-deoxy-6ξ, 9α-epoxyprostaglandin F_{1α} 60 methyl ester (500 mg) was stirred with methanolic NaOMe prepared from Na (0.23 g., 10 equivs.) and MeOH (3.5 ml.) under N₂ at room temperature overnight; 1N aq. NaOH (2.5 ml.) was added to the yellow reaction solution to bring about hydrolysis of the ester moiety and, after 2 hours, the methanol was evaporated in vacuo at room temperature. The residual aqueous

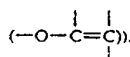
solution gave rise spontaneously to a mass of colourless fine needles of the desired sodium salt (formula (I): R=Na) which was cooled (0°), collected, washed sparingly with 1N aq. NaOH, air-dried, and stored in a stoppered tube; this salt (383 mg.) had max (KBr disc) 1692 cm⁻¹



and twenty ¹³C resonances only were observed (at 182.7 (C-1), 158.2 (C-6), 140.0 and 134.3 (C-13, 14), 100.7(C-5), 87.5(C-15), 80.6 and 75.5(C-9, 11), 58.0(C-12), 49.0, 45.8, 42.4, 41.9, 37.5, 35.8 (C-18), 31.6., 29.9, 29.3, 26.7(C-19), and 18.4(C-20) ppm from TMS in DMSO-d₆). The product, sodium (5Z)- 5,6-didehydro-9-deoxy-6,9α-epoxyprostaglandin F_{1α}(syn. sodium prostacyclin), thus obtained completed inhibited arachidonic acid-induced platelet aggregation (human platelet-rich plasma) at 1 ng./ml. and its profile of biological activity on the rabbit aorta, rabbit coeliac artery, rat stomach strip and rat colon conformed with that of sodium prostacyclin obtained by biosynthesis. After air-drying, the salt has a surface coating of sodium carbonate (ca 3.5% by weight) which protects the vinyl ether moiety against carbondioxide catalysed hydrolysis.

EXAMPLE 8

5ξ-Iodo-9-deoxy-6ξ,9α-epoxyprostaglandin F_{1α} 65 methyl ester was treated with 1,5-diazabicyclo-5-nonene (DBN) at room temperature in the absence of a solvent for a few hours. The DBN and hydrogen iodide were conveniently removed by adsorption on to a column of SiO₂, prepared from a suspension of SiO₂ in EtOAc/Et₃N 50:1, and the vinyl ether was eluted with the same solvent system. I.R. spectroscopy (thin film, ν 1738 (CO₂Me) and 1696 cm⁻¹



¹H n.m.r. in C₆D₆-Et₃N, 19:1 (δ4.22, triplet of triplets¹², J_{6,9} and 1.0 Hz (C-5 vinyl proton)), and ¹³C n.m.r. in C₆D₆-Et₃N, 19:1 (distinctive features were resonances at 159.8 (C-1), 155.8 (C-6), 137.2 and 130.6 (C-13,14), 95.3 (C-5), 84.1 (C-15) 77.3 and 72.2 (C-9,11) and 51.1 (Me ester)p.p.m. from TMS).

The vinyl ether (5Z)-5,6-didehydro-9-deoxy-6,9α-epoxyprostaglandin methyl ester, was hydrolysed with aqueous sodium hydroxide to give sodium prostacyclin (formula (I): R=sodium).

What we claim is:

1. Prostacyclin substantially free from other biological material.

2. The method of treating thrombosis in a mammal which comprises administering an effective antithrombotic portion of prostacyclin to said mammal in need thereof.

3. The method of inhibiting the aggregation of blood platelets in a mammal which comprises administering an effective platelet antiaggregation amount of prostacyclin to said mammal.

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4. A method of preventing thrombosis in a mammal in need thereof which comprises administering an effective thrombosis prevention amount of prostacyclin to said mammal.

5. A method for the prevention or treatment of gastric lesions in a mammal comprising the administration of a non-toxic prevention or treatment amount of prostacyclin to said mammal.

6. A method for the treatment of a wound in a mammal which comprises administering to said mammal a non-toxic wound treatment amount of prostacyclin.

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7. A method for producing a vasodilatory action in the blood vessels of a mammal in need thereof comprising the administration of an effective vasodilatory amount of prostacyclin to said mammal.

8. The method of preparing (5-Z)-5,6-Didehydro-9-deoxy-6,9 α -epoxyprostaglandin F_{1 α} which comprises incubating microsomes of mammalian tissue with a prostaglandin endoperoxide.

9. The method of claim 8 in which the microsomes are aortic microsomes and the prostaglandin endoperoxides are PGG₂ or PGH₂.

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EXHIBIT 11

U.S. PATENT 4,338,325

United States Patent [19]

Johnson et al.

[11] 4,338,325

[45] Jul. 6, 1982

[54] PGI₂ PHARMACOLOGICALLY
ACCEPTABLE SALTS

[75] Inventors: Roy A. Johnson, Kalamazoo; Frank
H. Lincoln, Portage; John E. Pike,
Kalamazoo, all of Mich.

[73] Assignee: The Upjohn Company, Kalamazoo,
Mich.

[21] Appl. No.: 200,690

[22] Filed: Oct. 27, 1980

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 819,940, Jul. 28, 1977,
which is a continuation-in-part of Ser. No. 725,550,
Sep. 22, 1976, abandoned, which is a continuation-in-
part of Ser. No. 716,770, Aug. 23, 1976, abandoned.

[51] Int. Cl.³ A61K 31/557; C07D 307/935
[52] U.S. Cl. 424/285; 549/465

[58] Field of Search 260/346.22, 346.73;
542/421; 424/285

[56] References Cited

U.S. PATENT DOCUMENTS

3,598,858 8/1971 Bergstrom et al. 562/503

OTHER PUBLICATIONS

Moncada et al., Prostaglandins, vol. 12, pp. 658-713,
(1976).

Primary Examiner—Henry R. Jiles

Assistant Examiner—Bernard Deniz

Attorney, Agent, or Firm—Robert A. Armitage

[57] ABSTRACT

The present invention relates to PGI₂ pharmacologi-
cally acceptable salts, having pharmacological activity.
Particularly, the compounds described herein are useful
as platelet aggregation inhibitors.

6 Claims, No Drawings

4,338,325

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PGI₂ PHARMACOLOGICALLY ACCEPTABLE SALTS

DESCRIPTION

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of co-pending application Ser. No. 819,940, filed July 28, 1977; which is a continuation-in-part of Ser. No. 725,550, filed Sept. 22, 1976, now abandoned; which was a continuation-in-part of Ser. No. 716,770, filed Aug. 23, 1976, now abandoned.

BACKGROUND OF THE INVENTION

The present invention relates to novel compositions of matter which are derivatives of prostacyclin or PGI₂. The chemical name for prostacyclin is (5Z)-5,6-dihydro-9-deoxy-6,9-epoxy-PGF₁α. Specifically the present invention relates to PGI₂ pharmacologically acceptable salts.

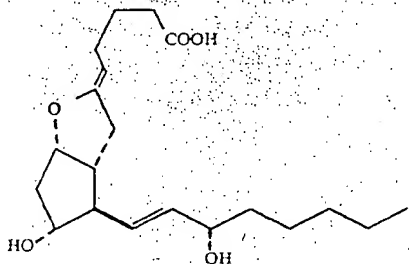
Prostacyclin itself was first reported as "PGX" by Moncada and his co-workers. See Moncada, et al., *Prostaglandins* 12:658-713 (1976). Prostacyclin is a circulating hormone in the arterial circulation of mammals. See "Prostacyclin As A Circulating Hormone", *Nature* 273:767-768 (June 29, 1978) and Gryglewski, R. J., et al., "Generation of Prostacyclin By Lungs In Vivo And Its Release Into Arterial Circulation," *Nature* 273:765-767 (June 29, 1978).

PRIOR ART

Subsequent to any invention described herein the existence of prostacyclin as a naturally-occurring composition of matter was reported in the aforementioned references.

SUMMARY OF THE INVENTION

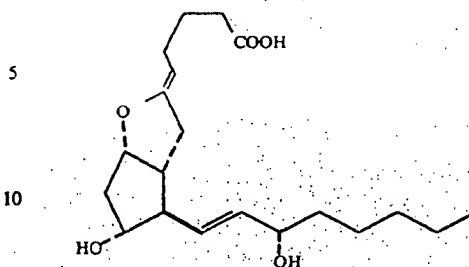
The present invention relates to a composition of matter consisting essentially of a pharmacologically acceptable salt of a compound of formula I



and

a parenteral pharmaceutical composition characterized in being prepared from

(1) a free flowing powder form of the sodium salt of PGI₂; a compound of formula I



and

(2) a conventional pharmaceutical diluent for parenteral formulations.

Compounds in accordance with the present invention are prepared by chemical methods and have pharmacological uses. Accordingly, compounds in accordance with the present invention are useful for a variety of pharmacological purposes. Accordingly, the compounds in accordance with the present invention are especially useful whenever it is desired to inhibit platelet aggregation, to reduce the adhesive character of platelets, and to remove or prevent the formation of thrombi in mammals, including man. For example, these compounds are useful in the treatment and prevention of myocardial infarcts, to treat and prevent post-operative thrombosis, to promote patency of vascular drafts following surgery, and to treat conditions such as atherosclerosis, arteriosclerosis, blood clotting defects due to lipemia, and other clinical conditions in which the underlying etiology is associated with lipid imbalance or hyperlipidemia. Other in vivo applications include geriatric patients to prevent cerebral ischemic attacks and long-term prophylaxis following myocardial infarcts and strokes. For these purposes, the compounds are administered systemically, e.g., intravenously, subcutaneously, intramuscularly, and in the form of sterile implants for prolonged action. For rapid response, especially in emergency situations, the intravenous route of administration is preferred. Dosages in the range about 0.01 to about 10 mg/kg of body weight per day are used, the exact dose depending on the age, weight, and condition of the patient or animal, and on the frequency and route of administration.

The addition of these compounds to whole blood provides in vitro applications, such as storage of whole blood to be used in heart-lung machines. Additionally, whole blood containing these compounds can be circulated through limbs and organs, e.g., heart and kidneys, whether attached to the original body, detached and being preserved or prepared for transplant, or attached to a new body. Blocking of aggregated platelets is avoided by the presence of these compounds. For this purpose, the compound is added gradually or in single or multiple portions to the circulating blood, to the blood of the donor person or animal, to the perfused whole body, attached or detached, to the recipient, or to two or all of those at a total steady state dose of about 0.001-1.0 μg/ml of whole blood. These compounds are also useful in preparing platelet-rich concentrates from blood for use in treating thrombocytopenia or in chemotherapy.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention especially provides for the preparation of compositions of matter consisting essentially of pharmacologically acceptable salts of prostacyclin. Most conveniently, these pharmacologically acceptable salts of prostacyclin are prepared from the corresponding methyl ester, PGI₂ methyl ester or (5Z)-9-deoxy-6,9α-epoxy-5,6-didehydro-PGF₁, methyl ester. The preparation of the methyl ester starting material is described in U.S. Pat. No. 4,158,667, incorporated here by reference (e.g., Example 7 therein). From the crystalline form of this methyl ester described in U.S. Pat. No. 4,158,667, compositions of matter consisting essentially of pharmacologically acceptable salts of prostacyclin are prepared in accordance with the following examples:

EXAMPLE 1

(5Z)-9-Deoxy-6,9α-epoxy-Δ⁵-PGF₁, sodium salt.

A mixture of 0.030 g (5Z)-9-deoxy-6,9α-epoxy-Δ⁵-PGF₁, methyl ester in 5 ml of methanol is treated with 9 ml of 0.01 N NaOH and stirred at about 250° C. for 72 hr. The solution is then concentrated, diluted with 5 ml of water, frozen at about -75° C. and lyophilized overnight. The title compound is obtained as a white free-flowing powder having infrared absorption at 3320, 1693, 1555, and 1470 cm⁻¹.

The procedure above is repeated using larger quantities. From 0.150 g of the enol ether methyl ester there is obtained 0.155 g of the title compound as a white free-flowing powder. A sample of the material dissolved in methanol-water shows practically no mobility by TLC on silica gel plates in acetone-dichloromethane (3:7), compared with the starting material which has R_f 0.45 (TLC on silica gel in acetone-dichloromethane (3:7) using plates pretreated in triethylamine-(5%)-dichloromethane).

Following the procedure of Example 1, but replacing sodium hydroxide with excess sodium carbonate suspended in water, there is obtained the title compound after 72 hr at about 25° C. The product is precipitated with acetonitrile.

EXAMPLE 2

(5Z)-9-Deoxy-6,9α-epoxy-Δ⁵-PGF₁, benzyltrimethylammonium salt.

A solution of 0.25 g of (5Z)-9-deoxy-6,9α-epoxy-Δ⁵-PGF₁, methyl ester in 5 ml of methanol is treated with one ml of water and 0.5 ml of methanolic solution (40%) of benzyltrimethylammonium hydroxide. The mixture is left standing at about 25° C. for 22 hr and then concentrated to one-fourth volume. The concentrate is made up with water to a volume of 25 ml, frozen with Dry Ice and lyophilized to yield the title compound, a solid, having infrared absorption at 3375, 1700, 1570, 1395, 1125, 1090, 1035, 975, 895, 780, 725, and 705 cm⁻¹.

EXAMPLE 3

(5Z)-9-Deoxy-6,9α-epoxy-Δ⁵-PGF₁, tetramethylammonium salt

A solution of 0.25 g of (5Z)-9-Deoxy-6,9α-epoxy-Δ⁵-PGF₁, methyl ester in 5 ml of methanol is treated with one ml of an aqueous solution (10%) of tetramethylammonium hydroxide, it thereafter following the procedure

of Example 2, to obtain the title compound, a solid, having infrared absorption at 3375, 1695, 1570, 1495, 1395, 1125, 1090, 1035, 970, and 955 cm⁻¹.

Accordingly, there is provided by the present invention novel compositions of matter consisting essentially of a single, pharmacologically acceptable salt of prostacyclin.

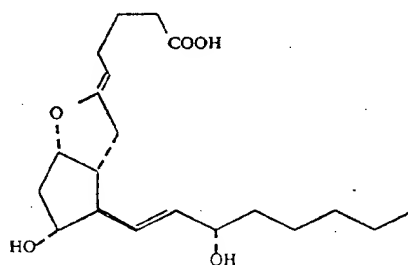
Accordingly, the present compositions of matter consisting essentially of a pharmacologically acceptable salt of prostacyclin are surprisingly and unexpectedly more useful than preparations of prostacyclin heretofore obtainable from biological sources in that the novel compositions of matter are

- (1) highly chemically homogeneous
- (2) more readily sterilized for parenteral administration,
- (3) more conveniently and elegantly formulated as pharmaceutical entities, and
- (4) more efficacious in obtaining prostaglandin-like biological effects.

Thus, the present invention provides compositions of matter surprisingly and unexpectedly more useful than forms of prostacyclin known prior to the present invention.

We claim:

1. A composition of matter consisting essentially of a pharmacologically acceptable salt of a compound of formula I



2. A composition according to claim 1 wherein said pharmacologically acceptable salt is the benzyltrimethylammonium salt.

3. A composition according to claim 1 wherein said pharmacologically acceptable salt is the tetramethylammonium salt.

4. A composition according to claim 1 wherein said pharmacologically acceptable salt is the sodium salt.

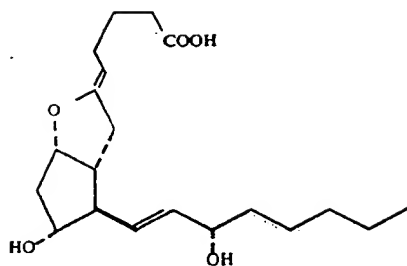
5. A composition according to claim 4 in a free flowing powder form.

6. A parenteral pharmaceutical composition for inhibition of platelet aggregation characterized in being prepared from

- (1) a free flowing powder form of the sodium salt of PGI₂, a compound of formula I

4,338,325

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1 and

(2) a conventional pharmaceutical diluent for parenteral formulations, such that the sodium salt of PGI₂ is present in said composition at a concentration sufficient to inhibit platelet aggregation upon administration of a said composition in a predetermined volume or at a predetermined rate.

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EXHIBIT 12

TERMINAL DISCLAIMER
FOR
U.S. 4,883,812

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Salvador Moncada

GROUP : 121

SER. NO. : 07/237,987

EXAMINER: Dentz, B.

FILED : August 29, 1988

FOR : ETHERS

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

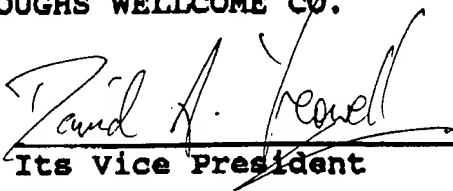
TERMINAL DISCLAIMER TO OBVIATE A DOUBLE
PATENTING REJECTION OVER A PRIOR PATENT

Your petitioner, BURROUGHS WELLCOME CO. residing at Research Triangle Park in the State of North Carolina represents that it is the owner of said application Serial No. 07/237,987, filed on the 29th day of October, 1988 for ETHERS. ITS ownership by way of an assignment is recorded in parent application Serial Number 795,524, filed May 10, 1977, now U.S. Patent 4,539,333 of which this application is a continuation, at Reel 3479, Frames 683-686, recorded on December 7, 1977. Your petitioner hereby disclaims the terminal part of any patent granted on the above-identified application, which would extend beyond the expiration date of the full statutory term as presently shortened by any terminal disclaimer of Patent No. 4,539,333, and hereby agrees that any patent so granted on the above-identified application shall be enforceable only for and during such period that the legal title to United States Patent No. 4,539,333, this agreement to run with any patent granted on the above-identified application and to be binding the grantee, its successors or assigns.

Petitioner does not disclaim any terminal part of any patent granted on the above-identified application prior to the expiration date of the full statutory term as presently shortened by any terminal disclaimer of Patent No. 4,539,333, in the event that it later expires for failure to pay a maintenance fee, is held unenforceable, is found invalid, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321(a), has all claims cancelled by a reexamination certificate, or is otherwise terminated prior to the expiration of its statutory term as presently shortened by any terminal disclaimer, except for the separation of legal title stated above.

BURROUGHS WELLCOME CO.

By:

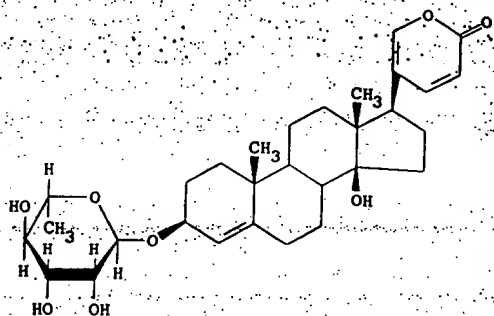

Its Vice President

6/13/89

EXHIBIT 13

ITEM 7890 MERCK INDEX
PROSTACYCLIN

20, 1 (1970). Metabolic studies: Davis et al., *Arch. Int. Pharmacodyn.* 177, 231 (1969); Nakano et al., *ibid.* 183, 199 (1970). Clinical studies: Several authors, *Minerva Med.* 67, 4243-4322 (1967).



Prisms from methanol, mp 219-222°, $[\alpha]_D^{20} -91.5^\circ$ (CH₂OH); LD₅₀ orally in male, female rats: 56, 76 mg/kg. E. I. Goldenthal, *Toxicol. Appl. Pharmacol.* 18, 185 (1971).

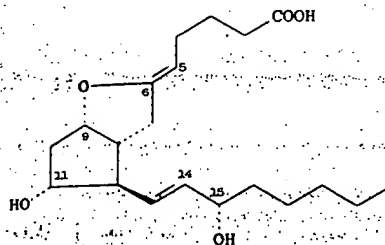
Proscillaridin-4'-methyl ether, C₃₁H₄₄O₈, *meproscillaridin*, Clift, mp 213-217°, $[\alpha]_D^{20} -94^\circ$ (CH₂OH); uv max (CH₂OH): 297 nm (log ϵ 3.79), (1N KOH/CH₂OH): 355 nm (log ϵ 4.65). Sol in methanol, ethanol, THF, dioxane; slightly sol in CHCl₃, CH₂Cl₂; acetone; insol in water, nonpolar organics. Series of articles on prepn, pharmacology, toxicology, pharmacokinetics, metabolism: *Arzneimittel-Forsch.* 28, 493-573 (1978).

THERAP CAT: Cardiotonic.

7890. Prostacyclin. (5Z,9 α ,11 α ,13E,15S)-6,9-Epoxy-11,15-dihydroxyprosta-5,13-dien-1-oic acid; (5Z)-9-deoxy-6,9 α -epoxy- Δ^5 -PGF_{1 α} ; epoprostenol; prostaglandin I₂; prostaglandin X; PGI₂; PGX; U-53217. C₂₀H₃₂O₅; mol wt 352.48. C 68.15%, H 9.15%, O 22.70%. A prostaglandin produced by enzymatic transformation of prostaglandin endoperoxides (GG₂, PGH₂), which dilates blood vessels and is approximately 30 times more potent than prostaglandin E₁, q.v., in inhibiting platelet aggregation. Evidence for its occurrence during biosynthetic conversion of arachidonic acid by rat stomach homogenates: C. Pace-Asciak, L. S. Wolfe, *Biochemistry* 10, 3657 (1971). Isolin from microsomes of pig and rabbit aorta by J. R. Vane and co-workers: S. Moncada et al., *Nature* 263, 663 (1976). PGI₂ is also synthesized in bovine coronary arteries as well as human arteries and veins: *idem*, *Lancet* 1, 18 (1977); G. J. Dusting et al., *Prostaglandins* 13, 3 (1977); by cultured human and bovine endothelial cells: B. B. Weksler et al., *Proc. Nat. Acad. Sci. USA* 74, 3922 (1977); by pig aortic endothelial cells: D. E. MacIntyre et al., *Nature* 271, 549 (1978). It has been suggested that endoperoxides released by platelets can be converted to PGI₂ by vascular tissue and that a balance between formation of PGI₂ and release of thromboxane A₂, q.v., which induces platelet aggregation, controls the formation of thrombi in blood vessels. It has also been postulated that PGI₂ acts to stimulate platelet adenylate cyclase and to prevent the action of thrombi on phospholipid breakdown as well as platelet aggregation. Structure: R. A. Johnson et al., *Prostaglandins* 12, 915 (1976). Synthesis: E. J. Corey et al., *J. Am. Chem. Soc.* 99, 3006 (1977); of sodium salt and stereochemistry: R. A. Johnson et al., *ibid.* 4182. Additional syntheses: I. Tomoskozi et al., *Tetrahedron Letters* 1977, 2627; N. Whittaker, *ibid.* 2805; K. Nicolaou, *Chem. Commun.* 1977, 630. Synthesis of the 5E-isomer: E. J. Corey et al., *Tetrahedron Letters* 1977, 3529. Chemical stability in aq solns: M. J. Cho, M. A. Allen, *Prostaglandins* 15, 943 (1978).

Biosynthetic study: V. Tomasi et al., *Nature* 273, 670 (1978). Biological properties: R. J. Gryglewski et al., *Prostaglandins* 12, 685 (1976). Preliminary clinical study: A. E. S. Gimson et al., *Lancet* 1, 173 (1980). Antimetastatic effects: K. V. Honn et al., *Science* 212, 1270 (1981); *idem*, *ibid.* 217, 542 (1982). Preliminary study of effect of PGN infusion in patients with acute myocardial infarction: O. Edhag et al., *N. Engl. J. Med.* 308, 1032 (1983). Review of biological properties: S. Moncada, J. R. Vane, *Clin. Sci.* 65,

369-372 (1981); of therapeutic potential: *idem*, *Advan. Pharmacol. Ther.* 4, 215-233 (1982); of physiological role: J. R. Vane et al., *Int. Rev. Exp. Pathol.* 23, 161-207 (1982). General reviews: S. Moncada, J. R. Vane, *Fed. Proc.* 38, 66-71 (1979); J. C. McGiff, *Ann. Rev. Pharmacol. Toxicol.* 21, 479-509 (1981); S. Moncada et al., *Advan. Pharmacol. Ther.* 6, 39-47 (1982). Books: *Prostacyclin*, J. R. Vane, S. Bergstrom, Eds. (Raven Press, New York, 1979) 453 pp; *Prostaglandins in Cardiovascular and Renal Function*, A. Scriabine et al., Eds. (Spectrum Publications, New York, 1980) 498 pp.

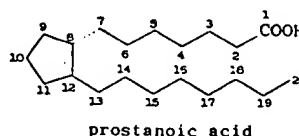


Chemically unstable in aq soln. Hydrolyzes to 6-oxo-PGF_{1 α} . Half-life at 4° is approx 14.5 min when total phosphate is 0.165 M. Anti-aggregating activity disappears within 0.25 min on boiling or within 10 min at 37°.

Sodium salt, C₂₀H₃₁NaO₅, U-53217A, *Cyclo-Prostin*, *Flolea*. Hygroscopic, free-flowing white powder. Stable for 2 months if kept dry at -30°.

THERAP CAT: Platelet aggregation inhibitor.

7891. Prostaglandin(s). A family of biologically potent lipid acids first discovered in seminal fluid and extracts of accessory genital glands of man and sheep: von Euler, *Arch. Exp. Pathol. Pharmacol.* 175, 78 (1934); *Klin. Wochenschr.* 14, 1182 (1935). Isolin: Bergstrom, Sjoval, U.S. pats. 3,069,322 and 3,598,858 (1962, 1971); Samuelsson, *J. Biol. Chem.* 238, 3229 (1963). Also found in lower concns in other organs: *idem*, *Biochim. Biophys. Acta* 84, 707 (1964). The single non-mammalian source of prostaglandin intermediates, or syntons, is the gorgonian sea whip or sea fan, *Plexaura homomalla*: Weinheimer, Spraggins, *Tetrahedron Letters* 1969, 5185; Schneider et al., *J. Am. Chem. Soc.* 94, 2122 (1972). Prostaglandins are named as derivatives of *prostanic acid*. Prostaglandins are divided into the types E, F, A, B, C, and D based on functions in the cyclopentane ring. Numerical subscripts refer to the number of unsaturations in the side chains; α or β subscripts refer to the configuration of substituents in the ring. Six naturally occurring prostaglandins, E₁, E₂, E₃, F_{1 α} , F_{2 α} , F_{3 α} , are considered primary in that no one is derived from another in the living organism. First structural and stereochemical elucidations: Bergstrom et al., *Acta Chem. Scand.* 16, 501 (1962); *idem*, *J. Biol. Chem.* 238, 3555 (1963). Absolute config: Nugteren et al., *Nature* 212, 38 (1966). First total synthesis of racemic PGE₁ and PGF_{1 α} : Corey et al., *J. Am. Chem. Soc.* 90, 3245 (1968). Review of synthetic studies: Pike, *Fortschr. Chem. Org. Naturst.* 28, 313 (1970); Axen et al., in *The Total Synthesis of Natural Products*, vol. 1, J. ApSimon, Ed. (Wiley-Interscience, New York, 1973) pp 81-143; Clarkin in *Progress in Organic Chemistry*, vol. 8, W. Carruthers, J. K. Sutherland, Eds. (Wiley, New York, 1973) pp 1-28. Book: J. S. Bindra, R. Bindra, *Prostaglandin Synthesis* (Academic Press, New York, 1977).



Biosynthesis occurs by enzymatic conversion of unsaturated twenty-carbon fatty acids. Review of biosynthetic studies: Samuelsson, *Progr. Biochem. Pharmacol.* 5, 109 (1969). Review of metabolism: Samuelsson et al., *Ann. N.Y. Acad. Sci.* 180, 138 (1971). Biological activities include stimulation of smooth muscle, dilation of small arteries, bronchial dila-

tion, lowering of blood pressure, of lipolysis, labor, abortion, a pressure. Implications reactions, nasal vasomotor, autonomic neurot and biochemical. (1965); Weeks, An man, *Ann. Rev. Biochem. Physiol.* 221, 1251 (1965). Preparation of prostaglandins: M. Landis, W. L. (1982) 705 pp. Gen (1967); Bergstrom Ramwell, Shaw, I Pike, *Sci. Amer.* 2, 410-487. *Prostaglandins in Drug Res.* 1973) pp 410-487. Ramwell, Ed. (P *Prostaglandins in Scriabine et al., F* 1980) 498 pp; Ca glandins, A. Herm 472 pp. Three v *Reproduction; Pro Aspects; Prostaglan Pathological Aspect sity Park Press, Ba*

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Crystals from e $[\alpha]_{578} -6.6^\circ$ (c = 1.0) in chloroform. THERAP CAT: Vas

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